Functional characterization of the ArcA and HlyX regulons of *Actinobacillus pleuropneumoniae*

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Erklärung zur Dissertation

Hiermit versichere ich an Eides statt, dass die Dissertation

Functional characterization of the ArcA and HlyX regulons of *Actinobacillus pleuropneumoniae*

selbständig verfasst und alle benutzten Hilfsmittel sowie evtl. zur Hilfeleistung herangezogene Institutionen vollständig angegeben wurden.

Die Dissertation wurde nicht schon als Diplom- oder ähnliche Prüfungsarbeit verwendet.

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(Unterschrift Falk Büttner)

Imagination is more important than knowledge,
for knowledge is limited while imagination embraces
the entire world

Albert Einstein
(1879 – 1955)

Meinen Eltern

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Zusammenfassung

Funktionelle Charakterisierung der ArcA- und HlyX-Regulons von *A. pleuropneumoniae*

Das gram-negative Bakterium *Actinobacillus* (*A*.) *pleuropneumoniae* ist der Erreger der Porcinen Pleuropneumonie. Die Anpassung der Genexpression an anaerobe Bedingungen ist eine virulenzassoziierte Eigenschaft von *A. pleuropneumoniae* und erfolgt durch HlyX (das FNR-Homolog von *A. pleuropneumoniae*) und das ArcAB Zweikomponentensystem. Ziel dieser Arbeit war die Identifizierung der ArcA- und HlyX- Regulons und deren Bedeutung für die Virulenz.

Das *arcA*-Gen von *A. pleuropneumoniae* wurde identifiziert, deletiert und die Mutante durch PCR, PFGE, Southern Blot und DNA-Sequenzierung überprüft. *A. pleuropneumoniae* Δ*arcA* zeigte weder einen Wachstumsdefekt noch ein reduziertes Überlebensvermögen. *A. pleuropneumoniae* bildete bei anaerobem Wachstum in Flüssigkultur Autoaggregate, die bei der *arcA*-Deletionsmutante nicht mehr zu beobachteten waren. Darüberhinaus war die Biofilmbildung der *arcA*-Deletionsmutante reduziert. Ein Tierversuch am Schwein ergab eine geringere Virulenz der *arcA*-Deletionsmutante gegenüber dem Wildtyp. Klinische Symptome, pathologische Lungenveränderungen und Reisolierbarkeit waren signifikant reduziert.

Um den Phänotyp von *arcA*- und *hlyX*-Deletionsmutante auf molekularer Ebene zu klären, wurden beide Mutanten mit Hilfe von „microarrays“ und durch differentielle zwei-dimensionale Gelelektrophorese (2D DIGE) untersucht. Die Aufklärung des ArcA-Regulons ergab, dass ArcA 93 Gene um mehr als 1,5-fach auf- und 106 Gene um mehr als 1,5-fach abreguliert; durch HlyX wird die Expression von 398 Genen um mehr als 1,5-fach auf- und von 505 Genen entsprechend abreguliert. Die Ergebnisse der Transkriptomanalyse wurden im Wesentlichen durch die Proteomanalysen bestätigt. Die Auswertung der von ArcA regulierten Gene impliziert, dass ArcA den Stoffwechsel auf Fumarat-Atmung unter anaeroben Bedingungen einstellt. In einem begleitenden Projekt konnte diese Annahme inzwischen gestützt werden. Die Deletion der Fumarat-Reduktase führte zu einer Attenuierung von *A. pleuropneumoniae*.

Ein Vergleich der „microarray“ Analysen ergab, dass ArcA und HlyX 64 Gene auf- und 39 Gene abregulierten. Eine gegensätzliche Regulation durch die beiden Transkriptionsfaktoren wurde bei 11 Genen festgestellt. HlyX agierte als starker Positiv-Regulator von terminalen Reduktasen, die DMSO, TMAO, Nitrat oder Nitrit verwenden. Während HlyX die Expression von 30 Genen um mehr als 7-fach aufregulierte, wurde für ArcA nur ein einziges Gen, das für eine putative Methylierungsuntereinheit eines Restriktions-Modifikationssystems kodiert, gefunden, dessen Expression so deutlich verstärkt wurde. Im Gegensatz dazu wurde kein Gen gefunden, das durch HlyX um mehr als 7-fach abreguliert wurde, während ArcA die Expression von 13 Genen um mehr als 7-fach reduzierte. Diese Ergebnisse führen zu dem Schluss, dass ArcA von *A. pleuropneumoniae* als starker Positiv-Regulator und dass HlyX als starker Negativ-Regulator der Transkription agieren. Dadurch wird erstmalig die Bedeutung beider Regulatoren gezeigt, sich unter anaeroben Bedingungen in ihrem Spektrum zu ergänzen.

Summary

Functional characterization of the ArcA and HlyX regulons of *A. pleuropneumoniae*

The gram-negative bacterium *Actinobacillus* (*A.*) *pleuropneumoniae* is the causative agent of Porcine Pleuropneumonia. Adaptation of gene expression to anaerobicity is a virulence-associated trait of *A. pleuropneumoniae* and is mediated by HlyX (the FNR homologue of *A. pleuropneumoniae*) and the ArcAB two-component system. The objective of this study was the identification of the HlyX and ArcA regulons of *A. pleuropneumoniae* and the characterization of both regulons with respect to their putative role in infection.

The *arcA* gene *of A. pleuropneumoniae* was identified and deleted, and the deletion mutant was confirmed by PCR, PFGE, Southern blot and nucleotide sequencing. The *arcA* deletion mutant exhibited no growth defect and aging was not affected. The characteristic autoaggregation of *A. pleuropneumoniae* wt upon growth under anaerobic conditions was abolished, and biofilm formation was reduced in the mutant. A pig infection experiment revealed that deletion of the *arcA* gene caused a severe attenuation. Thus, scores determined for clinical symptoms, lung lesion and bacterial reisolation were significantly reduced.

In order to investigate the phenotypic characteristics of the *A. pleuropneumoniae arcA* and *hlyX* deletion mutant an in-depth analysis of the ArcA regulon was performed using microarrays and 2D DIGE. Investigation of the ArcA regulon revealed that the expression of 93 genes was upregulated by ArcA more then 1.5-fold and that expression of 106 genes was downregulated. HlyX upregulated expression of 398 genes and downregulated expression of 505 genes. By 2D DIGE and subsequent mass spectrometry the results of the array analyses were mostly confirmed. Analysis of the ArcA regulon implies that *A. pleuropneumoniae* anaerobically adapts its metabolism in order to use fumarate as a terminal electron acceptor for respiration. This hypothesis could be supported in an accompanying project. Thus, deletion of the fumarate reductase of *A. pleuropneumoniae* caused a significant attenuation in infection.

Comparison of microarray results revealed that 64 genes were up- and 39 genes were downregulated by both ArcA as well as HlyX whereas 11 genes were identified as being regulated in opposite directions. HlyX acts as a positive regulator for terminal reductases using DMSO, TMAO, nitrate and nitrite as substrates. HlyX increases the expression of 30 genes by 7-fold or more, compared to a single gene encoding a putative methylation subunit of a type III restriction-modification system, upregulated to that extend by ArcA. In contrast, no gene is repressed by HlyX by more then 7-fold compared to 13 genes that are repressed by ArcA more then 7-fold. These findings lead to the conclusion that ArcA of *A. pleuropneumoniae* is primarily acting as a strong transcriptional repressor whereas HlyX mostly acts as a strong transcriptional inducer. This mode of regulation underlines the supplementary functions of ArcA and HlyX during anaerobiosis when both factors are active.

Schlagworte

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Table of contents

List of abbreviations

A. (bi)dest.	Aqua (bi)destillata
A. pleuropneumoniae	*Actinobacillus pleuropneumoniae*
ABTS	2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate]
bp	base pair(s)
BCIP	5-bromo-4-chloro-3-indolyl phosphate
cDNA	complementary DNA
CFU	colony forming unit(s)
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidin triphosphate
dGTP	deoxyguanosin triphosphate
dTTP	deoxytymidin triphosphate
Da	Dalton
DEPC	diethyl pyrocarbonate
2D DIGE	two-dimensional difference gel electrophoresis
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
DTT	dithiotreitol
E. coli	*Escherichia coli*
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme linked immunosorbent assay
et al.	et alii
Fig.	figure
g	grams or gravity
h	hours
H. influenzae	*Haemophilus influenzae*
IEF	isoelectric focussing
IgG	immunoglobulin G
IPG	immobilized pH gradient
IPTG	isopropyl-β-D-thiogalactopyranoside
k	kilo
kb	kilo base pair(s)
kDa	kilo Dalton
l	liter
LB	Luria Bertani
L. monocytogenes	*Listeria monocytogenes*
LPS	lipopolysaccharide
M	molar
m	milli

µ	micro
MALDI-TOF-MS	matrix assisted laser desorption / ionisation time-of-flight mass spectrometry
Mb	mega base pair(s)
min	minute(s)
ml	millilitre
mRNA	messenger RNA
MS	mass spectrometry
mW	molecular weight
n	nano
NAD	nicotinamide adenine dinucleotide
NBT	nitroblue tetrazolium
OD_{xxx}	optical density at xxx nanometers
ORF	open reading frame
p	pico
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
pI	isoelectric point
PMSF	phenylmethylsulfonyl fluoride
Q-TOF MS	quadrupole time-of-flight mass spectrometry
®	registered trademark
RNA	ribonucleic acid
RNase	ribonuclease
rpm	rotations per minute
RT	room temperature
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
sec	seconds
S. Typhimurium	*Salmonella* Typhimurium
S. aureus	*Staphylococcus aureus*
TCA	trichloroacetic acid
TCA cycle	tricarboxylic acid cycle
TMAO	trimethyl amine N-oxide
TOF	time-of-flight
UV	ultraviolet
U	unit
V	volt
v/v	volume per volume
w/v	weight per volume
wt	wild type
TM	trade mark
2D-PAGE	two-dimensional-polyacrylamide gel electrophoresis

A Introduction

Actinobacillus (*A*.) *pleuropneumoniae* is a gram negative rod belonging to the family of *Pasteurellaceae*. This facultative anaerobe is a highly host specific, strictly extracellular pathogen that causes Porcine Actinobacillus Pleuropneumonia (PAP). PAP is a respiratory disease of fattening pigs occurring worldwide and causing significant economic losses. The natural infection in pigs begins with inhaling of *A. pleuropneumoniae*-containing aerosols originating from infected animals. Then, *A. pleuropneumoniae* colonizes the respiratory epithelium and tonsils and, when reaching the lower respiratory tract, causes a variety of symptoms. These range from peracute death over acute pleuropneumonia with necrotic and hemorrhagic lesions to development of chronic disease. Subclinical infections also occur. A problem in controlling PAP is the occurrence of clinical healthy carrier animals. These animals can carry and shed the pathogen for up to several months.

Several virulence or virulence-associated factors such as the Apx toxins (*A. pleuropneumoniae* specific RTX-toxins), transferrin-binding proteins or capsule have been identified. New virulence-associated factors like DMSO reductase, aspartate ammonia lyase, and HlyX (the FNR analogue of *A. pleuropneumoniae*) were discovered recently implying an important role of anaerobic metabolism for virulence and persistence of *A. pleuropneumoniae*. In its natural niche, the porcine respiratory tract, *A. pleuropneumoniae* has to adapt to an anaerobic or reducing environment. However, adaptation to anaerobiosis at the molecular level and its contribution to virulence and persistence have not been investigated in *A. pleuropneumoniae* to date.

Therefore the aim of this study was the identification of genes controlled by the major oxygen-dependent transcriptions factors ArcA and HlyX. The ArcA and HlyX regulons should then provide insights into the mechanisms of anaerobic adaptation of *A. pleuropneumoniae* relevant *in vivo*. This knowledge of the molecular adaptation to anaerobiosis should then result in the identification of new virulence-associated factors or pathways.

B Literature Review

B 1 *Actinobacillus pleuropneumoniae*

B 1.1 Taxonomy

Actinobacillus (*A*.) *pleuropneumoniae* is a gram negative rod that belongs to the family of *Pasteurellaceae*. It was originally classified as *Haemophilus* (*H*.) *pleuropneumoniae* (Shope et al., 1964). Due to its high homology to *A. lignieresii* on the DNA level the species was transferred to the genus *Actinobacillus* (Pohl et al., 1983). *A. pleuropneumoniae* strains can be subdivided into two biotypes based on their ability to synthesize nicotine amide adenine dinucleotide (NAD). The biotype 1 is NAD-dependent whereas biotype 2 is not NAD-auxotrophic (Pohl et al., 1983; Nicolet 1992; Nielsen et al., 1997). Twelve serotypes for biotype 1 and six for biotype 2 are known to date, based on discrimination by differences in their surface polysaccharides (Bosse et al., 2002). The *A. pleuropneumoniae* biotype 1 serotypes 1 and 5 are subdivided into subtypes a and b, respectively. Three additional serotypes (13, 14 and 15) have been proposed more recently (Nielsen et al 1997; Blackall et al., 2002).

B 1.2 Infection and disease

Actinobacillus pleuropneumoniae was first identified as the causative agent of porcine pleuropneumonia in Great Britain in 1957 (Nicolet 1992). It is highly host specific for pigs although a singular isolation from lambs has been described (Nielsen 1986; Hervas et al., 1996). *A. pleuropneumoniae* occurs worldwide but the serotypes are not equally distributed. Serotypes 1, 5, and 7 are more prevalent in the United States, serotypes 1, 3, and 5 appear mostly in Canada. Serotypes 1, 2, 3, 5, and 9 are more frequent in Europe (Blaha 1992, Chiers et al., 2002).

Infection with *A. pleuropneumoniae* is typically caused by inhalation of droplets at close range (Nicolet et al., 1969) and can occur in shared air space or due to direct contact with infected pigs (Taylor, 1995; Torremorell et al., 1997; Jobert et al., 2000). *A. pleuropneumoniae* has low tenacity and is not able to survive in the environment for prolonged periods of time. Therefore transmission via personnel rarely occurs (Fenwick and Henry 1994). Persistently infected clinically healthy carrier animals that are introduced into *A. pleuropneumoniae*-free herds are the major source for severe outbreaks (Rycroft and Garside 2000). *A. pleuropneumoniae* causes significant economic losses due to death in

acute disease on the one hand and reduced growth rates of convalescent pigs on the other hand (Straw et al., 1989).

Pigs of all age groups can be infected but 10 to 16 week old animals are most susceptible (Fenwick and Henry 1994). The inhalation of *A. pleuropneumoniae*-containing aerosols can cause an infection with symptoms ranging from peracute to chronic depending on serotype, infectious dose, and immune status of the affected animal (Sebunya et al., 1983; Rogers et al., 1990; Cruijsen et al., 1995). Peracute and acute disease is generally accompanied by clinical symptoms like fever, dyspnoea, tachypnoea, anorexia and vomitus. The pathogen often causes fibrinous and necrotizing pleuropneumonia, sometimes associated with pulmonary haemorrhage, and affects pericardium and joints. In the chronic state, lung lesions appear as sequestered abscesses with persistent pleural adhesions (Matschullat 1983; Bertram 1985; Liggett et al., 1987; Didier et al., 2002). Convalescent and latently infected pigs showing no symptoms can still harbour *A. pleuropneumoniae* and thereby serve as reservoirs (Liggett et al., 1987; Fenwick and Henry 1994).

B 1.3 Virulence factors

Virulence factors are defined as bacterial products that facilitate growth or survival of a pathogen in the host, contributing to infection and disease (Mekalanos 1992; Mahan et al., 1996). Genes encoding for metabolic enzymes can contribute either to virulence or to general bacterial functions outside the host. These factors are designated as virulence-associated factors.

RTX toxins

Known virulence factors of *A. pleuropneumoniae* are the RTX toxins that occur also in many other gram negative bacteria (Welch 1991). The RTX toxins are secreted by type I secretion systems and are capable of lysing erythrocytes (Thompson et al., 1993). The genome of *A. pleuropneumoniae* encodes four RTX toxins that have been designated ApxI to ApxIV. These toxins are differently distributed among the different serotypes (Schaller et al., 1999).

Type IV fimbriae

Type IV fimbriae are multifunctional bacterial surface organelles expressed by most gram negative bacterial pathogens. *Actinobacillus pleuropneumoniae* encodes an intact gene cluster for the synthesis of type IV fimbriae. Fimbriae have been shown for *A. pleuropneumoniae* to mediate adherence to swine alveolar epithelial cells *in vitro* (Van

Overbeke et al., 2002), and the fimbrial promoter (Tfp) was identified as upregulated upon contact to cultured epithelial cells (Boekema et al., 2004).

LPS and capsule

The major adhesin of *A. pleuropneumoniae* has been identified as lipopolysaccharide (LPS, Belanger et al., 1990). LPS is exposed at the surface and binds to porcine respiratory tract cells (Paradis et al., 1994) and to porcine haemoglobin (Belanger et al., 1995).

A further virulence factor of *A. pleuropneumoniae* is its capsule. The capsule protects *A. pleuropneumoniae* from bactericidal activity of serum and was identified to be antiphagocytic (Inzana et al., 1988). Comparison between virulent and non-virulent *A. pleuropneumoniae* isolates revealed a more distinct and adherent capsule in the virulent isolate. Additionally, the virulent isolate contained more LPS (Jensen and Bertram 1986).

Iron acquisition

The availability of iron is very low for bacterial pathogens within a mammalian host. Due to the poor solubility of ferric iron on the one hand and its association to iron scavenging host proteins on the other hand there is virtually no free iron available to support bacterial growth. *A. pleuropneumoniae,* like other members of the families *Neisseriaceae* and *Pasteurellaceae,* has developed a sophisticated iron uptake system which allows the utilization of transferrin-bound iron (Perkins-Balding et al., 2004; Gonzalez et al., 1990; Gerlach et al., 1992). Additionally hemoglobin (Belanger et al., 1995; Archambault et al., 2003; Srikumar et al., 2004) and hemin (Deneer and Potter 1989; Archambault et al., 2003; Srikumar et al., 2004) can serve as iron sources. Mutants lacking transferrin-binding proteins are avirulent and unable to colonize, underlining the role of transferrin uptake for *A. pleuropneumoniae* (Baltes et al., 2002).

Biofilm formation

Biofilm formation is a virulence-associated trait of many bacterial pathogens and has been shown to be prevalent among field isolates of *A. pleuropneumoniae* (Kaplan, Mulks 2005). Biofilms are composed of bacterial cells embedded into an extracellular polysaccharide matrix. The *A. pleuropneumoniae* biofilm matrix has been described as a linear polymer of N-acetyl-D-glucosamine residues in β(1,6) linkage, called PGA which also functions as the biofilm matrix in other phylogenetically diverse bacteria. The gene locus *pgaABCD* encodes the enzymes responsible for production of PGA in *A. pleuropneumoniae*. PGA itself is the substrate for dispersin B, a biofilm releasing glycosyl hydrolase also encoded on the *A. pleuropneumoniae* genome (Kaplan et al., 2004).

Anaerobic metabolism

Deletion of genes encoding enzymes (DMSO reductase, aspartate ammonia lyase) or regulators (HlyX) for anaerobic metabolism of *A. pleuropneumoniae* did not affect aerobic growth *in vitro* but reduced virulence and persistence of *A. pleuropneumoniae* in a pig infection experiment (Baltes et al., 2005; Baltes et al., 2003; Jacobsen et al., 2005). Identification of genes expressed in *A. pleuropneumoniae* during the chronic state of infection revealed several genes that have been associated with virulence in *A. pleuropneumoniae* or other bacteria. An autotransporter serine protease of *A. pleuropneumoniae* has been identified as expressed *in vivo* in the chronic state of infection and is positively regulated under anaerobic conditions by the global regulator HlyX (Baltes and Gerlach 2004a; Baltes et al., 2007).

The porcine respiratory tract is a well aerated and strongly supplied with blood providing, on first sight, aerobic conditions for growth of *A. pleuropneumoniae*. However, infection with *A. pleuropneumoniae* is often accompanied by severe tissue destruction and leads to the formation of purulent encapsulated sequesters. This necrotic tissue is separated from the airways and, in addition, poorly supplied with blood. Therefore it appears likely that these sequesters are oxygen-deprived providing microaerophilic or anaerobic conditions. However, deletions in *A. pleuropneumoniae* genes involved in anaerobic metabolism not only reduced the ability to survive within these sequesters. The bacteria were also attenuated with respect to survival on intact lung epithelium in pig infections experiments (Baltes et al.; 2005, Baltes et al., 2003; Jacobsen et al., 2005). A possible reason for this observation could be the presence of the airway reductant glutathione that has been found in elevated levels upon infection of mice with *Pseudomonas aeruginosa* (Cantin et al., 1987; Day et al., 2004).

B 2 Mechanism for redox control of gene expression

B 2.1 Overview

Bacterial gene expression in response to alterations in redox potential is controlled by several regulatory proteins that use different mechanism. The sulphur atoms of FNR cysteine residues build a [4Fe-4S] cluster under reducing conditions that mediates dimerisation and thereby DNA binding activity of FNR. The SoxR / SoxS regulatory proteins regulate gene expression in response to superoxide and provide defence against oxidative damage. SoxR forms a homodimer containing two stable [2Fe-2S] centres. These become oxidized and thereby activated when challenged with oxidative stress conditions. FixL is a histidine sensor kinase using heme as a cofactor for binding of oxygen. Thereby autophosphorylation activity

is affected. NifL contains FAD as a redox-responsive cofactor. Under oxidizing conditions NifL binds and inactivates NifA, a transcriptional activator of nitrogen fixation genes. A further transcription factor, OxyR, responds to the redox potential by breaking or forming a disulfide bond that affects its DNA-binding activity. ArcB is a transmembrane histidine kinase that reacts to reducing conditions by autophosphorylation. Then it activates ArcA, a global anaerobic transcription factor. The regulator of anaerobic gene expression in α-purple proteobacteria, RegB, senses respiratory activity of cytochrome oxidase. A redox-responsive cysteine residue seems to be the redox sensor in CrtJ, the aerobic repressor of photopigment synthesis. The FNR protein and the ArcAB two-component system play the major role for the general adaptation of metabolic activity to anaerobiosis (Bauer et al., 1999).

B 2.2 The ArcAB two-component system of bacteria

Two-component signal transduction systems are common in prokaryotes and play important roles in adaptation to environmental changes (Hoch and Silhavy 1995). The Arc (anoxic redox control) two-component system allows facultative anaerobic bacteria to sense reducing conditions and to adapt their gene expression accordingly (Lynch and Lin 1996; Iuchi and Lin 1995). The *arcA* gene has been initially designated as *dye*, because its deletion renders *E. coli* sensitive to the dye toluidine blue. *E. coli* Δ*dye* was not able to form F pili resulting in a sterile strain (Buxton and Drury 1983); the respective gene had been identified before and designated as *sfrA* (Beutin et al., 1981). In 1988 ArcA was identified as a negative regulator for enzymes associated with aerobic metabolism during anaerobiosis, like i) several dehydrogenases of the flavoprotein class, ii) cytochrome c oxidase complex, and iii) members of the tricarboxylic acid cycle, the glyoxylate shunt, and the pathway for fatty acid degradation (Iuchi and Lin 1988). One year later the second pleiotropic control gene for anaerobic metabolism, *arcB*, was identified at a different position on the chromosome. Deletion of *arcB* in *E. coli* also caused sensitivity to toluidine blue and relieved anaerobic expression of genes for aerobic metabolism (Iuchi et al., 1989). This led to the description of the Arc two-component system consisting of the cytosolic response regulator ArcA and a transmembrane histidine sensor kinase, ArcB (Iuchi et al., 1990c). As a typical response regulator ArcA possesses an N-terminal receiver domain with a conserved Asp residue at position 54 and a C-terminal helix-turn-helix DNA binding domain. The sensor kinase ArcB exhibits two transmembrane domains that are connected by an unusually short periplasmic domain of only 16 amino acid residues (Kwon et al., 2000). ArcB contains three catalytic domains, namely an amino terminal transmitter domain, a central receiver domain and a

carboxy terminal phosphotransfer domain (Iuchi et al., 1990c; Ishige et al., 1994). Under reducing conditions, ArcB is autophosphorylated and then transphosphorylates ArcA via a [His-292 → Asp-576 → His-717]$_{ArcB}$ → [Asp-54]$_{ArcA}$ phosphorelay (Georgellis et al.,1997; Kwon et al., 2000). The direct signal that silences the kinase activity of ArcB under aerobic conditions of growth is the interaction with oxidized quinone electron carriers (Georgellis et al., 2001). The molecular mechanism of kinase silencing involves the oxidation of two cytosol-located redox-active cysteine residues participating in intermolecular disulfide bond formation (Fig. 1; Malpica et al., 2004).

B 2.3 FNR

The FNR protein was initially identified in the mid 1970s by Guest and coworkers by isolation and characterization of mutants not able to perform fumarate and nitrate reduction (Lambden and Guest 1976). FNR has been shown to activate the expression of anaerobic respiratory enzymes that utilize alternative terminal electron acceptors like TMAO/DMSO, fumarate or nitrate (Cotter and Gunsalus 1989; Jones and Gunsalus 1987; Stewart 1982). Several aerobic respiratory enzymes like cytochrome d and cytochrome o oxidase (Cotter et al., 1990) and NADH dehydrogenase (Spiro et al., 1989) are repressed by FNR under anaerobic growth conditions. FNR activates transcription by interacting with the α-subunit of the RNA polymerase (Williams et al., 1997). FNR and the catabolite activator protein (CAP) share striking sequence similarities (Shaw et al., 1983). Different to CAP, however, FNR contains a ferredoxin-like four cysteine cluster (Cys-X_3-Cys-X_2-Cys-X_5-Cys). Three out of four cysteine residues in this cluster and a fourth cysteine residue about 100 amino acids away are required for FNR activation; this led to the hypothesis that FNR senses the redox potential due to an iron centre coordinated by four cysteine residues (Green et al., 1993; Melville and Gunsalus 1990; Sharrocks et al., 1990; Spiro and Guest 1988). FNR has been shown to contain, under anaerobic conditions, one $[4Fe\text{-}4S]^{2+}$ cluster per subunit. These $[4Fe\text{-}4S]^{2+}$ clusters are highly labile and rapidly disassemble when exposed to oxygen resulting in a $[2Fe\text{-}2S]^{2+}$ cluster. This cluster is more stable to oxygen but unable to sustain biological activity (Khoroshilova et al., 1997; Lazazzera et al., 1996). When the cells are shifted back to anaerobic conditions the $[4Fe\text{-}4S]^{2+}$ cluster is regenerated. Prolonged incubation (hours) with oxygen results in complete disassembly of the $[2Fe\text{-}2S]^{2+}$ cluster and in formation of the apoprotein that lacks iron (Popescu et al., 1998). Under anaerobic conditions FNR is a dimer with specific DNA binding activity. The Fe-S cluster is disrupted by oxygen and FNR is converted into the inactive monomeric form (Fig. 1; Lazazzera et al., 1996).

An *A. pleuropneumoniae* protein that conferred hemolytic activity to *E. coli* was identified as very similar to the *E. coli* FNR protein and designated as HlyX. Although HlyX induced a hemolytic phenotype in *E. coli* it exhibits no similarity with known hemolysins or cytotoxins. Functional homology between *E. coli* FNR and *A. pleuropneumoniae* HlyX could also been shown by HlyX mediated complementation of the nutritional lesion of an *E. coli* FNR mutant. The hemolytic activity in *E. coli* caused by expression of HlyX is supposed to be a regulatory rather then a direct effect (Frey et al., 1989; Lian et al., 1989; McInnes et al., 1990). HlyX of *A. pleuropneumoniae* induced expression of the hemolysin in *E. coli* only under anaerobic conditions. Additionally, HlyX activated expression of the fumarate reductase subunit A in an *E. coli fnr* deletion mutant (Soltes and McInnes 1994). Similar to FNR, HlyX can acquire a [4Fe-4S] cluster that promotes binding to the FNR box under anaerobic conditions. Furthermore, HlyX and FNR have distinct but overlapping regulons in *E. coli* caused by an enhanced activating contact between HlyX and RNA polymerase at class I promoters (Green and Baldwin 1997) and, therefore, HlyX is considered the FNR analogue of *A. pleuropneumoniae*.

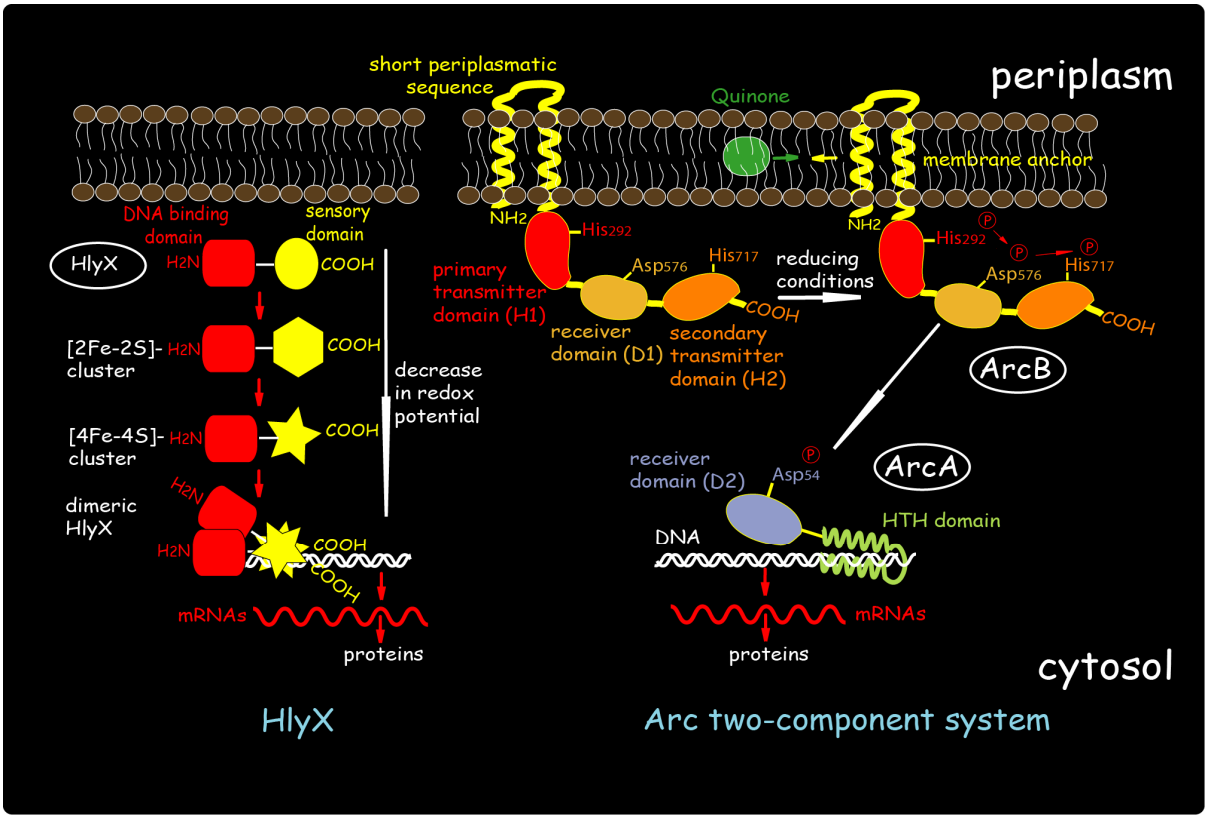


Fig. 1: HlyX and the Arc two-component system. HlyX and ArcA are transcription factors that become activated under reducing conditions. If no other oxidants (for example nitrate or nitrite) are available then reducing conditions develop by oxygen deprivation. Both systems use cysteine residues for redox sensing. The direct signal for HlyX is likely to be oxygen itself whereas the redox state of quinone electron carriers is the signal for ArcB.
This cartoon was made using the Adobe® Illustrator® CS software.

C Material and Methods

In this chapter parts were adopted from earlier PhD theses performed in Prof. Gerlach's laboratory.

C 1 Bacterial cultures

C 1.1 Bacterial strains, plasmids, and primers

Table 1: Bacterial strains and primers used in this study.

Strain, plasmid or primer	Characteristic(s)	Source and/or reference
Strains		
E. coli DH5α F´	F´ *endA1 hsdR17* ($r_K^-m_K^-$) *supE44 thi-1 recA1 gyrA* (Nal^r) *relA1* Δ(*lacZYA-argF*)*U169 deoR* [Φ80d*lac*Δ(*lacZ*)M15]	Raleigh et al., 1989
E. coli β2155	*thrB1004 pro thi strA hsdS lacZ*ΔM15 (F´ *lacZΔM15 laqI^q traD36 proA$^+$ proB$^+$*)Δ*dap*::*erm* (Erm^r) *recA*::*RPA-2-tet* (Tc^r)::Mu-km (Km^r) λ *pir*	Dehio and Meyer 1997
E. coli TOP10	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) Φ80*lacZ*ΔM15 Δ*lacX74 recA1 deoR araD139* Δ(*ara leu*) *7697 galU galK rpsL* (Str^r) *endA1 nupG*	TOPO TA cloning Invitrogen, Groningen, The Netherlands (Shuman, 1994)
A. pleuropneumoniae wt	*A. pleuropneumoniae* serotype 7 isolate 76	(Anderson et al., 1991)
A. pleuropneumoniae Δ*hlyX*	Unmarked *hlyX*-negative knockout mutant of *A. pleuropneumoniae* AP76	Baltes et al., 2005
A. pleuropneumoniae Δ*arcA*	Unmarked *arcA*-negative deletion mutant of *A. pleuropneumoniae* wt	This work
Plasmids		
pCR2.1-TOPO	*E. coli* cloning vector for fast and efficient cloning of *Taq* polymerase-amplified PCR products	TOPO TA cloning, Invitrogen, (Shuman, 1994)
pARC800	pCR2.1-TOPO based plasmid containing a 683-bp PCR product obtained with primers oArcA3 and oArcA4, starting at position 276 of the *arcA* gene and ending 221 bp downstream of the *arcA* stop codon	This work
pARC810	pCR2.1-TOPO based plasmid containing a 622-bp PCR product obtained with primers oArcA1 and oArcA2, starting 505 bp upstream of the *arcA* start codon and ending at position 88 of the *arcA* gene	This work
pARC820	pCR2.1-TOPO based plasmid obtained by digestion of pARC800 with EcoRI and BsmBI and ligation of the resulting 728-bp fragment into pARC810 digested with EcoRI and BsmBI	This work
pARC840	pCR2.1-TOPO based plasmid containing a 1715 bp PCR product obtained with primers oArcA9 and oArcA8, starting at position 996 bp upstream of the *arcA* start codon and ending at position 2 bp downstream the *arcA* stop codon	This work
pARC850	pCR2.1-TOPO based plasmid containing a 1236 bp PCR product obtained with primers oArcA1 and oArcA8, starting at position 505 bp upstream of the *arcA* start codon and ending at position 2 bp downstream the *arcA* stop codon	This work
pEMOC2	Transconjugation vector based on pBluescript SK with *mobRP4*, a polycloning site, Cm^r, and transcriptional fusion of the *omlA* promoter with the *sacB* gene	Accession no. AJ868288 (Baltes et al., 2003)
pARC700	pEMOC2 based plasmid carrying the truncated *arcA* ORF excised from	This work

	pARC820 using NotI and PspOMI and ligated into the pEMOC2 restricted with NotI and PspOMI	
pLS88	Broad-host-range shuttle vector from *Haemophilus ducreyi*; Str^r Sm^r Km^r	Willson et al., 1989
pHLYX1300	pLS88-based complementation plasmid containing *hlyX* ORF in a 5´-3´ orientation with respect to the vector derived *sulII* promoter	Baltes et al., 2005
pARC1320	pLS88-based complementation plasmid harboring the PCR product generated from primers oArcA9 and oArcA8 containing *arcA* ORF and additional 996 bp upstream of the *arcA* gene start codon, ligated into plasmid pLS88 after EcoRI restriction in a 3´-5´ orientation with respect to the vector derived *sulII* promoter	This work
pARC1340	pLS88-based complementation plasmid carrying the PCR product of primers oArcA1 and oArcA8 encoding the *arcA* ORF and 505 bp upstream sequence cloned into the EcoRI restriction site.	This work
Primers		
oArcA1	5´ TAACGCGGCCGCCCGCAAGAACGTATTTGGCG 3´;forward primer containing an internal NotI site (underlined) comprising positions 505-486 upstream of the *arcA* gene start codon	This work
oArcA2	5´ AATCCGTCTCTCCAAAACTTCATAACCTTCCGCCTCG 3´; reverse primer containing an internal BsmBI site (underlined) comprising positions 88-69 of the *arcA* gene	This work
oArcA3	5´ GATCCGTCTCTTTGGAAATCGGTGCGGACG 3´; forward primer containing an internal BsmBI site (underlined) comprising positions 276-295 of the *arcA* gene	This work
oArcA4	5´ ATCGGGGCCCACGACTAACGCAGGACCGGT 3´; reverse primer containing an internal PspOMI site (underlined) comprising positions 202-221 downstream of the *arcA* gene stop codon	This work
oArcA5	5´ CGCGCAATACGCTCAAAAGT 3´; forward primer comprising positions 44-63 of the *arcA* gene	This work
oArcA6	5´ CCGGTGTATTAGGGTGATCT 3´; reverse primer comprising position 660-641 of the *arcA* gene	This work
oArcA8	5´ TACTACTCTAACTCGCCGCA 3´; reverse primer comprising position 2 downstream the *arcA* stop codon to position 700 of the *arcA* gene	This work
oArcA9	5´ GAAATTGAGTCGCCGAGCTA 3´; forward primer comprising position 996-977 upstream of the *arcA* gene start codon	This work

[a] Erm^r, erythromycin resistance; Tc^r, tetracycline resistance; Km^r, kanamycin resistance, Str^r, streptomycin resistance; Sm^r, sulfonamide resistance

C 1.2 Growth conditions, media and supplements

E. coli strains were cultured in Luria-Bertani medium (10 g peptone [Roth, Karlsruhe, Germany], 5 g yeast extract [Roth], 5 g NaCl ad 1000 ml) supplemented with the appropriate antibiotics (ampicillin, 100 µg/ml [Roth], chloramphenicol, 25 µg/ml [Roth]) at 37°C; for cultivation of *E. coli* β2155 (Δ*dapA*), diaminopimelic acid (1 mM; Sigma Chemical Company, Deisenhofen, Germany) was added. *A. pleuropneumoniae* parent and mutant strains were cultured at 37°C and 5% CO_2 in PPLO medium (Difco, Augsburg, Germany), DMEM low glucose medium (# 31885, Invitrogen, Karlsruhe, Germany) or on PPLO agar (Difco) supplemented with NAD (10 µg/ml; Merck AG, Darmstadt, Germany), L-cysteine

hydrochloride (260 µg/ml; Sigma), L-cystine dihydrochloride (10 µg/ml; Sigma), and dextrose (1 mg/ml; Roth). The aerobic culture grown for experimental aerosol infection in addition contained Tween®80 (0.1%). For selection of *A. pleuropneumoniae* transconjugants (single crossing-overs) chloramphenicol (5 µg/ml; Roth) was added, and the medium for counter selection was prepared as described previously (Tonpitak et al., 2002). For cultivation of the complemented mutant kanamycinsulfate (25 µg/ml; Roth) was added. For anaerobic growth supplemented medium (PPLO or DMEM) was preincubated 48 h prior to inoculation in an anaerobic chamber (DonWhitley Scientific, Shipley, England) in an atmosphere containing 5% CO_2, 10% H_2, and 85% N_2 at 37°C. This medium was then inoculated with 1% of an aerobically grown log phase culture in supplemented PPLO medium with an optical density at 600 nm (OD_{600}) of 0.3. Anaerobiosis of the medium was confirmed using a dissolved oxygen sensor (CellOx® 325; WTW, Weilheim, Germany) linked to an inoLab® instrument (WTW, Weilheim, Germany). For recording of growth curves bacteria were harvested at 2, 4, 6, 8, 10 and 24 h post inoculation by centrifugation. As *A. pleuropneumoniae* showed heavy clumping upon growth under anaerobic conditions the protein content of whole-cell lysates was determined. Statistical analyses were performed using the Student's T-test. For RNA and protein preparations the bacteria were grown anaerobically in supplemented PPLO medium for 6 h and then harvested by centrifugation. For identification of secreted proteins bacteria were grown for 24 h in supplemented DMEM medium and then removed by centrifugation.

C 2 Manipulation of DNA

DNA modifying enzymes were purchased from New England Biolabs (Bad Schwalbach, Germany) and used according to the manufacturer's instructions. Taq polymerase was purchased from Gibco-BR Life Technologies (Karlsruhe, Germany). Chromosomal and plasmid DNA preparations, PCR, Southern blotting, transformation, and gel electrophoresis were done according to standard procedures (Sambrook et al., 1989), and pulsed field gel electrophoresis (PFGE) was performed as described previously (Oswald et al., 1999).

C 3 Construction of an isogenic *A. pleuropneumoniae arcA* in-frame deletion mutant

A 622-bp fragment containing the upstream part of the *arcA* gene with the flanking sequence was amplified from genomic *A. pleuropneumoniae* AP76 DNA by PCR using

primers oArcA1 and oArcA2 (Table 1), which contained a NotI (oArcA1) and a BsmBI (oArcA2) restriction endonuclease site and cloned into pCR2.1 TOPO, resulting in pARC810. In plasmid pARC810 the orientation of the upstream fragment is in opposite orientation to the interrupted *lacZ* gene. A 683-bp fragment containing the downstream part of the *arcA* gene with the flanking sequence was amplified from genomic *A. pleuropneumoniae* AP76 DNA using primers oArcA3 and oArcA4 (Table 1), which contained a BsmBI (oArcA3) and a PspOMI (oArcA4) restriction endonuclease site and cloned into pCR 2.1 TOPO resulting in pARC800. The 728-bp downstream fragment was removed from pARC800 using HindIII and BsmBI and cloned into plasmid pARC810 restricted with HindIII and BsmBI to obtain pARC820. Plasmid pARC820 harbours the truncated *arcA* sequence missing 186 bp of coding sequence. Plasmid pARC820 was restricted with NotI and PspOMI and the 1264-bp fragment was ligated into the conjugation plasmid pEMOC2 restricted with NotI and PspOMI resulting in plasmid pARC700. Plasmid pARC700 was used in the single-step transconjugation system (Oswald et al., 1999) to construct *A. pleuropneumoniae* ∆*arcA*. Colonies with the correct PCR profile were confirmed by Southern blot analysis (Sambrook et al., 1989) using the PCR product obtained with primers oArcA5 and oArcA6 from *A. pleuropneumoniae* ∆*arcA* as a probe, and by nucleotide sequence analysis (Seqlab, Göttingen, Germany). The absence of genomic rearrangements was confirmed by pulsed-field gel electrophoresis (PFGE).

C 4 Complementation of *A. pleuropneumoniae* ∆*arcA*

A 1236 bp fragment containing the entire open reading frame (ORF) of the *arcA* gene of *A. pleuropneumoniae* AP76 and 505 bp upstream sequence likely including regulatory motives was amplified by PCR using primers oArcA1 and oArcA8 (Table 1). A 1715 bp fragment was obtained by PCR using *A. pleuropneumoniae* AP76 genomic DNA and primers oArcA9 and oArcA8 (Table 1). This PCR product harbours 996 bp of *arcA* upstream sequence likely including regulatory sequences crucial for *arcA* expression. Both PCR products were cloned into pCR2.1 TOPO, restricted with EcoRI, and ligated into EcoRI restricted plasmid pLS88 yielding: i) pARC1340 (containing the 1236 bp fragment in a 5´-3´ orientation with respect to the vector-derived *sulII* promoter [positive orientation]), and ii) pARC1320 (containing the 1715 bp fragment in a 3´-5´ orientation with respect to the vector-derived *sulII* promoter [negative orientation]). These plasmids were electroporated into *A. pleuropneumoniae* ∆*arcA*. Functional complementation was tested as described below.

C 5 Determination of malic enzyme activity of *A. pleuropneumoniae*

The malic enzyme activity was measured spectrophotometrically at 340 nm by determination of NADPH formation (Geer et al., 1979). The assay buffer contained 35 mM Tris-HCl (pH 7.4), 3.3 mM L-malic acid (Roth), 0.3 mM NADP (Fluka, Seelze, Germany) and 5 mM $MnCl_2$ (Sigma). The reaction was initiated by the addition of whole-cell lysates of cultures of *A. pleuropneumoniae*, and the increase in absorbance at 340 nm was determined. Malic enzyme activity was expressed in units, with 1 U being the amount that converts 1.0 µmol of L-malate and NADP into pyruvate, CO_2 and NADPH per minute at pH 7.4 and 25°C. A molar extinction coefficient of 6.22 $mM^{-1}cm^{-1}$ at 340 nm was used to calculate the activity, and statistical analyses were performed using the Student's T-test .

C 6 Virulence studies

Virulence of *A. pleuropneumoniae* Δ*arcA* constructed in this study was examined in an aerosol infection model, mimicking the natural route of infection, in pigs 7 to 9 weeks of age.

C 6.1 Challenge experiment timeline

Day -7: -Arrival at the facility, blood samples taken for enzyme linked immunosorbent assay (ELISA)
Day -1: Clinical examination (including determination of body temperature)
Day 0: Clinical examination (including determination of body temperature);
Aerosol infection
Day 1-7: Clinical examination (including determination of body temperature)
Day 7: Collection of bronchoalveolar lavage fluid (BALF) from wt infected group;
Taking of blood samples and euthanasia of 4 pigs of Δ*arcA* infected group
Day 21: Taking of blood samples and euthanasia of 4 pigs of Δ*arcA* infected group
Taking of blood samples and euthanasia of 8 pigs of wt infected group

C 6.2 Origin and housing of the animals

Sixteen outbred pigs, 8 to 9 weeks of age were purchased from an *A. pleuropneumoniae*-free herd (no clinical symptoms and no serological responses in the ApxII-ELISA [Leiner et

al., 1999] and the deELISA [Goethe et al., 2000]) and randomly assigned to the different groups and cared for in accordance with the principles outlined by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Treaty Series, No. 123 [http://conventions.coe.int/Treaty/EN/Treaties/Html/123.htm; ETS No. 170]). The two groups were housed in separate isolation units with controlled temperature and ventilation.

C 6.3 Aerosol infection chamber

Infections were carried out in an aerosol infection chamber built by Impfstoffwerk Dessau Tornau GmbH (Dessau, Germany) based on the descriptions of Jacobsen et al. (Jacobsen et al., 1996). This chamber allows the simultaneous infection of four to five pigs 7-12 weeks of age. The top of the chamber consists of an acrylic window allowing easy surveillance of the animals during aerosol exposure. The chamber has two air vents equipped with filters, one of which is connected to a compressor (KNF Neuberger, Freiburg, Germany) used to exchange the air in the chamber. All tubing is made from either autoclavable silicone or Teflon. The bacterial suspension is aerosolized via a nozzle (Model no. 97058, Schlick Duesen, Untersiemau, Germany) operated by compressed air (Linde, Hannover, Germany).

C 6.4 Preparation of bacteria for aerosolization

For aerosol infection, 45 ml supplemented PPLO medium was inoculated with 5 ml of an overnight liquid culture of the respective *A. pleuropneumoniae* strain. The resulting culture was then incubated for approximately 2 hours at 37°C to OD_{600} of about 0.4. The culture was placed on ice, diluted 1:300 in ice-cold NaCl (150 mM), and kept on ice until use for a maximum of 2 hours. Immediately prior to aerosolization, bacteria were further diluted 1:100 in ice-cold NaCl (150 mM) resulting in a total living cell count per 13 ml dose (for four pigs) of approximately 1 x 10^5/ml; upon aerosolization, this dose corresponds to approximately 1 x 10^2 *A. pleuropneumoniae* cells per liter of aerosol in the chamber, a dose which has been titrated for the *A. pleuropneumoniae* strain AP76 to induce severe but not fatal disease (Teutenberg-Riedel et al., unpublished data).

C 6.5 Aerosol infection

Four pigs were challenged simultaneously within the chamber. In order to achieve an even distribution of the aerosol in the chamber, the nozzle was set to “5” and the valve regulating the flow of the fluid was set to “75”. The challenge dose of 13 ml diluted bacterial suspension was aerosolized using a pressure of 2 bar within a time of approximately 2 minutes. Ten minutes after complete aerosolization, the air in the chamber was exchanged ten times over duration of 20 min using a compressor. Then the pigs were led back to their stable.

C 6.6 Surveillance of the animals during the experiment

Clinical examinations were performed daily or as needed. Body temperature and clinical symptoms were recorded daily for each individual pig in the day before and up to 7 days after infection or as needed. A clinical scoring system based on the directive from the European Pharmacopoeia for the testing of *A. pleuropneumoniae* vaccines (porcine actinobacillosis vaccine [inactivated]) was employed to asses the clinical condition of each individual animal as follows. A score of 1 was given for each symptom including to the occurrence of coughing, dyspnoea, and vomitus, resulting in a minimum clinical score of 0 and a maximum score of 3 per day; the added daily clinical scores of day 1 to 7 were designated as the total clinical score. Statistical analysis of the total clinical score was performed using the Wilcoxon Signed-Rank Test.

C 6.7 Determination of lung lesion scores

Pigs were euthanized by intravenous injection of 10 ml pentobarbital (Eutha 77®) per pig. Lung lesions caused by *A. pleuropneumoniae* infection were determined and scored as described by Hannan et al. (Hannan et al., 1982) and statistically analyzed using the Wilcoxon Signed-Rank Test. Each lobe was separately scored. Dependent on the extent of pathological alterations a score from 0 (healthy) to 5 (complete pathological) was given for each lobe (resulting in a maximum score of 35 for the entire lung). This scoring system has been adopted in the European Pharmacopoeia (http://www.pheur.org) as the reference method in vaccine trials for *A. pleuropneumoniae*.

C 6.8 Bacteriological examination of organ samples, semi quantitative

The bacteriological examination included surface swabs of palatine tonsils, bronchial lymph nodes, and defined positions located in the outer third of each of the seven lung lobes; an additional swab of diseased lung tissue was taken if it was not covered by any of the defined lung locations. Plating was done on Columbia Sheep Blood agar to exclude other bacterial infections, as well as on selective meat blood agar (Jacobsen and Nielsen 1995), and fractionated twice. A score for reisolation of 0 was given for either no growth or if *A. pleuropneumoniae* colonies grew only in the directly swabbed area; a score of 1 was given if colonies were present in the fractionated streaks. The reisolation score was determined by adding theses numbers for each pig in the respective group, and the arithmetic means and standard deviations were determined. Several individual *A. pleuropneumoniae*-like colonies were subcultured on supplemented PPLO agar and confirmed by urease assay (Jacobsen et al., 2005) and PCR analysis using primers oArcA5 and oArcA6.

C 6.9 Enzyme Linked Immunosorbent Assay (ELISA)

The humoral immune response of pigs was determined by two different ELISAs. Antibody levels directed against the *A. pleuropneumoniae* ApxII toxin were analysed using an ELISA with ApxII as the solid phase antigen (Leiner et al., 1999). Antibodies directed against outer membrane components were determined with an ELISA using the detergent extract of *A. pleuropneumoniae* grown under iron restriction as the solid phase antigen (Goethe et al., 2000). The detergent extract was diluted 1:50 in carbonate buffer (50 mM; pH 9.6) and used for the coating of Polysorp® 96-microwell plates (Nunc, Rosklide, Denmark). Coating was performed with 100 µl for 16 h at 4°C. Plates were washed with PBST (8 g NaCl, 0.2 g KH_2PO_4, 1.44 g Na_2HPO_4, 0.2 g KCl, 0.5 ml Tween 20, ad 1000 ml) before the addition of serum, conjugate, and chromogen. Sera were initially diluted 1:100 and further twofold dilutions were performed in the plates in PBST. On each plate an internal positive control (pool of sera taken from pigs three weeks post infection with *A. pleuropneumoniae*) and a negative control (pool of sera taken from pigs prior to infection) was used. Serum dilutions and goat anti-pig peroxidase conjugate were each incubated for 1 h at room temperature. The ELISA was developed using 2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate] (ABTS) as a substrate. The test was considered valid when the OD_{405} of the negative serum at a 1:100 dilution was lower then the OD_{405} of the positive serum a 1:12,800 dilution. The titer is given by the serum dilution with an OD_{405} higher then twice the OD_{405} of the negative control serum at a 1:100 dilution.

C 7 Autoaggregation, electron microscopy and biofilm assays

A. pleuropneumoniae was grown in supplemented liquid medium under anaerobic conditions for six hours. Then cultures were removed from the anaerobic chamber and transferred on ice. For photography the liquid cultures were transferred into petri dishes. To perform raster electron microscopy these anaerobically grown cultures were transferred onto 0.2 µm membrane filters (Schleicher & Schuell, Dassel, Germany) and fixed with 2 N dimethylarsinic acid and 25 % (w/v) glutaraldehyde. Electron microscopy was performed at 10,000 fold enlargement (raster electron microscopy was performed by Armgard Janczikowski from the Leibniz University Hannover).

For the investigation of biofilm formation 20 ml of supplemented liquid PPLO medium in 200 ml glass Erlenmeyer flasks were inoculated with colony material from an agar plate and incubated for 6 h, 12 h, 18 h or 24 h at 37°C and 5% CO_2 without shaking. After incubation the OD_{600} was measured. The bacterial suspension was removed and the flasks were washed rigorously with water before staining. Then 2 ml of a 0.3% crystal violet solution (Merck) were added and the flasks were incubated for 5 min with gentle agitation. After staining the flasks were washed rigorously with water and then dried. The crystal violet-stained biofilm on the glass surface was solubilised with 10 ml of a solution comprised of 50% ethanol, 30% acetone and 20% acetic acid. The OD_{600} was determined and, using a calibration curve, the amount of crystal violet bound by the biofilm was calculated. All strains were tested in a minimum of four independent experiments. The flasks used were new and intensively cleaned and washed with distilled water between experiments. Statistical analyses were performed using the Student's T-test.

C 8 Microarray analysis

Preparation of CyDye™ labelled cDNA (C 8.2), microarray hybridization (C 8.3) and scanning of array slides was performed under supervision of Janine Bosse at the Imperial College of Science, London

C 8.1 RNA extraction

Bacteria were grown and harvested as described above. Bacterial RNA isolation was carried out using the FastRNA® Pro Blue Kit (Qbiogene) according to the manufacturer's instructions. RNA was further purified using the RNeasy Mini-Kit (QIAGEN, Hilden, Germany)

according to the manufacturer's recommendations. DNA contaminations were removed using the TURBO DNA-free™ Kit (Ambion, Austin, USA). RNA concentration was determined spectrophotometrically, and, quality and integrity was confirmed by agarose gel electrophoresis.

C 8.2 Preparation of CyDye™ labelled cDNA

Synthesis of cDNA and microarray hybridizations were performed as described previously according to the indirect labelling protocol adapted from Hughes et al., 2001 with some modifications. Briefly, 10 µg of total RNA in 11 µl water containing 3 µg random primers was heated at 70 °C for 5 min and then snap-cooled on ice. Then reverse transcription and simultaneous labelling followed in 25 µl 1x first strand synthesis buffer (10 mM dithiothreitol, 460 µM deoxinucleside triphosphate mix [dATP, dGTP, dTTP], 184 µM desoxycytidin triphosphate, 1.7 µl CyDye™ labelled deoxycytidin triphosphate [Amersham] and 500 units of Superscript II [Invitrogen]). The reaction was incubated in the dark for 10 min at 25 °C followed by 90 min at 42 °C. For each strain RNA was prepared from six independent cultures; three biological repeats each were labelled with Cy3 and three with Cy5 in order to avoid dye-related effects.

C 8.3 Microarray hybridization

The microarrays used in this study contained PCR products representing each of the 2025 ORFs of the genome of *A. pleuropneumoniae* serotype 5b strain L20 (Deslandes et al., 2007). Details on construction and content of the microarray (AppChip1) are available on the Institute for Biological Sciences website (http://ibs-isb.nrc-cnrc.gc.ca/glycobiology/appchips_e.html). Equivalent amounts of Cy3- and Cy5 labelled samples from *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* or A*. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX* were pooled and purified using the MinElute Purification Kit (Quiagen) according to the manufacturer's instructions. The purified Cy3 / Cy5 labelled samples were then added to the hybridization solution (4x SSC and 0.3% SDS). The hybridization solution was heated at 95°C for 2 min, slightly cooled and applied to the microarray. Hybridization was performed at 65°C in the dark for 16 to 20 h under glass cover slips in a high humidity chamber. The microarrays were then washed at 65°C for 2 min in 1x SSC and 0.05% SDS followed by two washing steps at RT for 2 min in 0.06x SSC. Slides were spun dry (1000 x g, 1 min) and stored in a light-tight box until scanning using a chip reader (GenePix 4000B, Axon

Instruments, now Molecular Devises, Union City, USA). Analysis of microarray data was performed using the software Genespring GX 7.3 (Agilent, Santa Clara, USA). In total six hybridizations were performed for i) six biological repeats of *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* and ii) six biological repeats of *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX*

C 9 Manipulation of proteins

C 9.1 Preparation of proteins

C 9.1.1 Preparation of whole cell lysates

Cultures were transferred on ice; bacteria were harvested by centrifugation (13,000 x g, 10 min, 4°C) and washed once with a buffer containing 10 mM Tris-HCl (pH 8.0) and 5 mM magnesium acetate. Pellets were resuspended in 50 mM Tris-HCl (pH 7.3) and stored at –70°C overnight. Cells were thawed, ruptured using the FastPrep® Instrument (Qbiogene) three times for 40 sec at intensity setting 5.0, and cell debris was removed by centrifugation (15,000 x g, 30 min). Protein concentration was determined with the MicroBC® assay (Uptima Interchim, Montlucon Cedex, France) and confirmed by SDS-PAGE.

C 9.1.2 Preparation of proteins of the inner and outer bacterial membrane

Whole cell lysates were ultracentrifuged (45000 rpm, SW55 Ti, 4°C, 2 h). The supernatant was removed and the pellet containing total membranes was resuspended in a solution containing 1% N-lauroylsarcosine (Sigma) and 1 mM β-mercaptoethanol (Sigma) and then incubated for 12 h at 4°C. The protein suspension was ultracentrifuged (45000 rpm, SW55 Ti, 4°C, 2 h); the pellet contained integral outer membrane proteins whereas inner membrane and outer membrane-associated whereas were enriched in the supernatant. The outer membrane pellet was solubilised in lysis buffer (30 mM Tris-HCl [pH 8.0], 7 M urea [Roth, Karlsruhe, Germany], 2 M thiourea [Sigma], and 4% [wt/vol] CHAPS [Roth]). The protein concentrations were estimated by SDS PAGE.

C 9.1.3 Enrichment of outer membrane-associated proteins by detergent wash

Upon 6 h of growth under anaerobic conditions chloramphenicol (Roth) was added to the cultures to a final concentration of 100 µg/ml. The cultures were incubated for further 10 min and then transferred on ice. Bacteria were harvested and washed as described above, resuspended in ice cold saline, one tablet complete protease inhibitor cocktail (Roche, Mannheim, Germany) chloramphenicol (Roth; 50 µg/ml [w/v]), sodium chloride (50 mM), Tris pH = 8 (10 mM), and sodiumdeoxycholate (0.05% [w/v], Sigma) were added, and cells were incubated with shaking for 1 h at 37°C. Finally cells and debris were removed by two centrifugation steps for 10 and 30 min respectively (13,000 x g, 4°C). Protein concentration of the supernatant was determined with the MicroBC® assay (Uptima Interchim, Montlucon Cedex, France) and confirmed by SDS-PAGE.

C 9.1.4 Preparation of secreted proteins

For preparation of secreted proteins *A. pleuropneumoniae* wt, *A. pleuropneumoniae* Δ*arcA* and *A. pleuropneumoniae* Δ*hlyX* were grown anaerobically in protein-free DMEM medium for 24 h upon 1% inoculation with an aerobic culture of OD = 0.3. The anaerobic cultures were cooled on ice and the cells were removed by two repeated centrifugations (13,000 x g, 10 min, 4°C). The protein concentrations were estimated by SDS PAGE.

C 9.2 One dimensional (1D) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed following standard procedures (Sambrook et al., 1989) using a Protean II Minigel system (BioRad, Munich, Germany). For quantitative 1D SDS-PAGE proteins were labelled with CyDyes™ (C 9.3.2) and scanning of fluorescently labelled proteins was performed with a Typhoon™ Trio scanner (Amersham, C 9.3.5). Quantitative analysis of band intensities was performed using the ImageQuant™ 5.2 software. Preparative gels were stained with either Coomassie blue G-250 (Roth) or colloidal Coomassie blue G-250 (Candiano et al., 2004). Bands of interest were cut from the gel, digested with trypsin (C 9.3.7) and analyzed by Q-TOF or MALDI TOF mass spectrometry (C 9.3.8)

C 9.3 Two-dimensional (2D) difference gel electrophoresis (DIGE)

C 9.3.1 Precipitation and reconstitution of proteins

2D DIGE experiments were performed for i) whole cell lysates, ii) inner membrane and outer membrane-associated proteins, iii) outer membrane-associated proteins, and iv) supernatant proteins. For each 2D DIGE experiment anaerobic growth of *A. pleuropneumoniae* wt, *A. pleuropneumoniae* ∆*arcA* and *A. pleuropneumoniae* ∆*hlyX* was repeated at least three times. Proteins were precipitated with TCA (10 to 20 %) over night and pelleted by centrifugation (15,000 x g, 30 min). The protein pellet was washed with acetone and solubilised in lysis buffer (30 mM Tris-HCl [pH 8.0], 7 M urea [Roth, Karlsruhe, Germany], 2 M thiourea [Sigma], and 4% [wt/vol] CHAPS [Roth]) to a protein concentration of 5 mg/ml. Insoluble material was removed by centrifugation at 16,000 x g for 5 min in a benchtop centrifuge.

C 9.3.2 Protein labelling with CyDye™

The pH of the protein solution was adjusted to 8.5 using 50 mM NaOH. The protein preparations of the wt and the mutant strains were labelled randomly with Cy3 or Cy5 (Amersham Biosciences AB, Uppsala, Sweden). Equal amounts of protein from each biological repeat of *A. pleuropneumoniae* wt and *A. pleuropneumoniae* mutant strains were pooled together as the internal standard and labelled with Cy2 (Amersham). Labelling was performed according to the manufacturer's instructions. Briefly, a 1 nmol/µl CyDye stock solution was prepared by dissolving 5 nmol CyDye™ in 5 µl dimethyl formamide (DMF [Sigma]). This stock solution was further diluted with DMF resulting in a 400 pmol/µl working dye solution. Theoretically 50 µg of protein had to be labelled with 400 pmol of Dye but practically the amount of dye could be reduced by up to 90% yielding in sufficiently labelled proteins. The protein solution was incubated for 30 min on ice in the dark with the respective dye. Then labelling was stopped by addition of 10 mM L-lysine (Sigma) to give a final concentration of about 0.2 mM L-lysine and then incubated for a further 10 min on ice in the dark. Labelled protein samples could now be stored at -70°C for at least 6 month.

C 9.3.3 First dimension focussing

The Cy2, Cy3 and Cy5 labelled protein preparations were pooled and then diluted with an equal volume of isoelectric focusing buffer (7 M urea [Roth], 2 M thiourea [Sigma], and 4% [wt/vol] CHAPS [Roth], 2 % (vol/vol) IPG buffer [Amersham], 2% [wt/vol] dithiothreitol [Roth]). Insoluble material was removed by centrifugation at 16000 x g for 5 min in a benchtop centrifuge. Immobiline DryStrips (24 cm, Amersham) were rehydrated with 450 µl of rehydration buffer per strip containing 2 M thiourea (Sigma), 7 M urea (Roth), 4% (wt/vol) CHAPS (Roth), 1% (vol/vol) IPG buffer (Amersham), and 0.2% (wt/vol) dithiothreitol (Roth) for 12-18 h using the Immobiline DryStrip Reswelling Tray (Amersham). Strips were transferred into the Ettan™ IPGphor™ Cup Loading Manifold (Amersham), and samples were loaded into anodal sample cups and subsequently focused using an Ettan™ IPGphor™ (Amersham) for 21 h in the following series of time blocks with increasing voltage: 3 h at 150 V, 3 h at 300 V, 6 h at a 1000 V gradient, 3 h at an 8000 V gradient, and 6 h at 8000 V. For second dimension, the strips were equilibrated twice for 15 min by rocking in a solution of 100 mM Tris-HCl (pH 8.0), 6 M urea (Roth), 30% (vol/vol) glycerol (Roth), and 2% (wt/vol) sodium dodecyl sulfate (SDS; Serva, Heidelberg, Germany) supplemented with 0.5% (wt/vol) DTT (Roth) for the first equilibration step and 4.5% (wt/vol) iodoacetamide (Sigma) for the second equilibration step.

C 9.3.4 Second dimension focussing

The Immobiline DryStrips (Amersham) were placed on the top of 12.5% polyacrylamide gels containing 12.5% (vol/vol) acrylamide (Serva) and 0.33% bisacrylamide (Serva) that had been precasted with low-fluorescent glass plates (Amersham) using an Ettan™ DALTsix gel caster (Amersham). The second-dimension SDS-PAGE was carried out at 12 °C and 50 V for 3 h followed by 100 V for 15 h using the Ettan™ DALTsix Electrophoresis System (Amersham).

C 9.3.5 Scanning of gels with fluorescently labelled proteins

The gels were scanned on a Typhoon Trio™ scanner (Amersham) at a scan resolution of 100 dots/cm using filters with excitation/emission wavelengths specific for Cy2 (filter: 520 BP 40; 488 nm / 520 nm), for Cy3 (filter: 580 BP 30; 532 nm / 580 nm), and for Cy5 (filter: 670

BP 30; 633 nm / 670 nm). The intensity of the pixel values was adjusted such that the maximum volume of each image was between 50,000 and 90,000.

C 9.3.6 Data analysis of 2D DIGE

Analysis of 2D DIGE was initially performed with the ImageQuant™ 5.2 software. Detailed and quantitative analysis was performed using the DeCyder™ 6.5 software (GE Healthcare) according to the manufacturer's instructions. Briefly, spot detection and matching of protein-spot maps of different gels was performed using the DeCyder™ biological variation analysis module (BVA). Detected and matched spots were also confirmed manually. Statistical analysis was performed using the unpaired T-test. Spots whose intensities were statistically significant ($p \leq 0.05$) different between the *A. pleuropneumoniae* wt group and the *A. pleuropneumoniae* Δ*arcA* group or between the *A. pleuropneumoniae* wt group and the *A. pleuropneumoniae* Δ*hlyX* group were selected for identification.

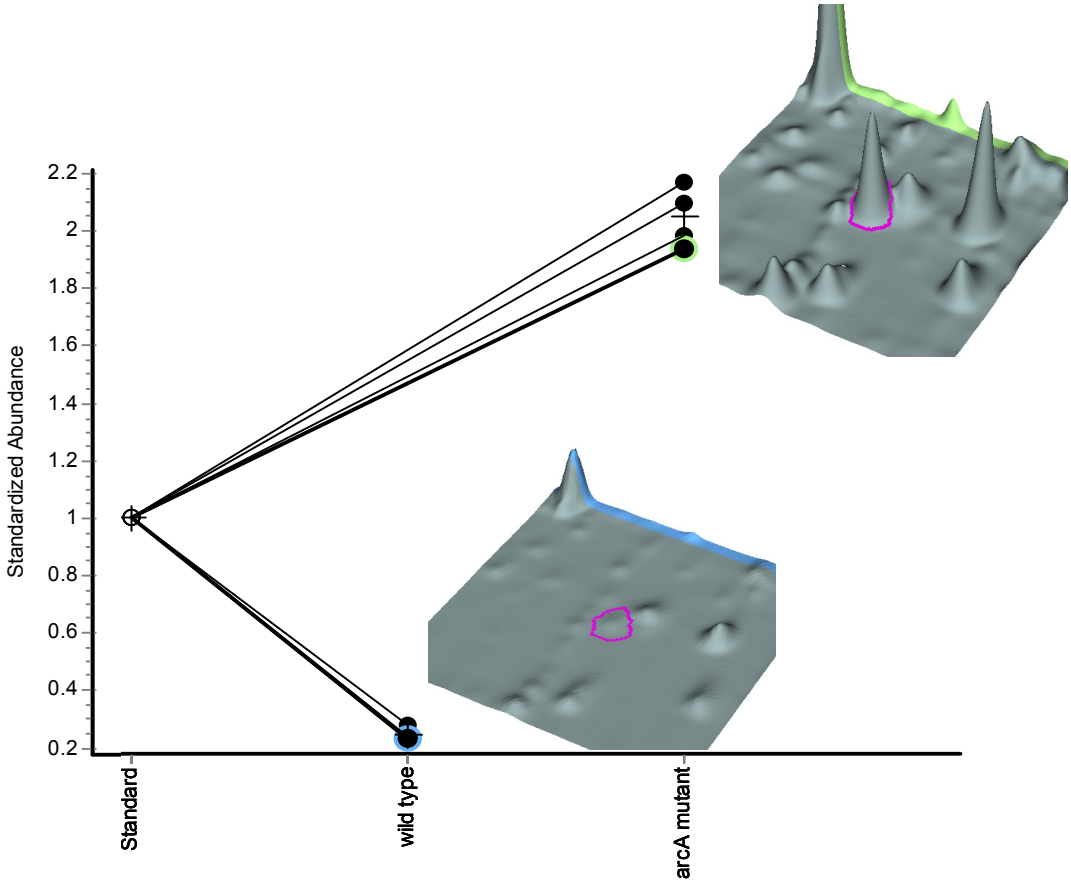


Fig. 2: Example of 2D DIGE data analysis using the Decyder™ 6.5 software. *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* were grown four times each under anaerobic conditions, independently. A protein preparation obtained from whole cell lysates was labelled with CyDyes™ and then separated by two-dimensional gel electrophoresis. Data analysis was performed using DeCyder™ 6.5 software. Here the standardized intensity is shown for an exemplarily protein for 4 biological repeats of *A. pleuropneumoniae* wt and for 4 biological repeats of *A. pleuropneumoniae* Δ*arcA*. The average ratio of the protein spots between wt and Δ*arcA* is -8.49. The respective protein is 8.49-fold increased in *A. pleuropneumoniae* Δ*arcA*. Statistical analysis by T-test revealed a p-vale of $2x10^{-08}$. By subsequent mass spectrometry this spot was identified as fructose-1,6-bisphosphatase.

Details:
Spot on 2D DIGE gel: Fpb (1) Fig. 12A on page 62
Ratio: Table 5 on page 69
Preparative gel for mass spectrometry: Gel 1 on page 160
Mass spectrometry: MS #2 Table Appendix G 6.1 on page 169

C 9.3.7 In-gel digestion

In order to pick spots that were identified by 2D DIGE, the analytical DIGE gels were either directly stained with colloidal Coomassie blue G-250 (Candiano et al., 2004) or preparative two-dimensional gel electrophoresis was performed loading 600 to 1500 µg protein per gel. Spots of interest were excised, the protein was trypsinated and removed from the gel according to the method of Wilm and Mann (Shevchenko et al., 1996). Briefly, the gel pieces were dehydrated with acetonitril (ACN, Merck), rehydrated with a 100 mM NH_4HCO_3 buffer containing 10 mM DTT (Roth), and then treated with 100 mM iodoacetamide (Sigma) in 100 mM NH_4HCO_3. Dehydration and rehydration were repeated, and then dehydrated gel pieces were rehydrated with buffer (50 mM NH_4HCO_3) containing 20 ng/µl trypsin (sequencing grade; Promega, Mannheim, Germany) and incubated for 12 - 16 h at 37°C. Peptides were extracted using 50 mM NH_4HCO_3 followed by extraction buffer containing 50% (vol/vol) ACN (Merck) and 5% (vol/vol) formic acid (Merck). The solution containing the extracted peptides was completely evaporated in a vacuum centrifuge. Peptides were redissolved in 3–5 µl of 50% (vol/vol) ACN (Merck), 0.1% (vol/vol) formic acid (Merck).

C 9.3.8 Protein identification by ESI Q-TOF MSMS or MALDI-TOF MS

For Electro-Spray Injection Quadrupole Time-of-Flight mass spectrometry (ESI Q-TOF MS) 3 µl of the peptide solution was loaded into a sample tip (Nanoflow Probe Tip – long, Waters, Milford, Massachusetts, USA). Peptide sequences were determined from tandem mass spectrometry (MS/MS) fragmentation data recorded on ESI Q-TOF mass spectrometer (Q-TOF Ultima, Waters) in positive reflection mode. Proteins were identified using the ProteinLynx Globals Server (Version 2.1, Waters) program by searching against the National Centre for Biotechnology Information (NCBI) complete database (ftp://ftp.ncbi.nlm.nih.gov./blast/db/FASTA/nr.gz) downloaded on 12.03.2007 or the *A. pleuropneumoniae* serotype 5b strain L20 genome database downloaded on 10.04.2007 (ftp://ftp.ncbi.nlm.nih.gov./genomes/Bacteria/Actino-bacillus_pleuropneumoniae_ L20/ NC_009053.faa). A background subtraction was performed with electrospray survey spectra but not with MSMS spectra. The Savitzky-Golay algorithm was applied for smoothing with 2 iterations. The smoothing window was set 3 channels. For deisotoping and centroiding the minimum peak width of electrospray data was set 4 channels. Centroid top was set at 80%, resolution at setting 10,000 and the NP multiplier at setting 1. For MSMS spectra deisotoping was performed (type: medium) using a threshold of 1%. For databank search a peptide tolerance of 100 ppm was set. The fragment

tolerance was set 0.1 Da with an estimated calibration error of 0.0025 Da. The molecular weight range was set from 0 to 400,000 Da and the pI range from 0 to 14. The minimum of peptides to match setting was set 1 and the maximum hits to return setting was 20. The primary digest reagent was set trypsin. One missed cleavage was allowed and fixed modifications were set as carbamidomethyl C, variable modification as oxidation M. An automatical validation of the results was performed.

Alternatively Matrix Assisted Laser Desorption / Ionization Time-of-Flight (MALDI-TOF) mass spectrometry (MS) was carried out on a Voyager-DE™ Pro (Applied Biosystems, Forster City, USA); 1 µl of the peptide solution was mixed with 1 µl of matrix (α-cyano-4-hydroxy-cinnamic acid [ACHC, Bruker Daltonics, Billerica, USA], 5 mg/ml in 50% ACN with 0.1% trifluoroacetic acid [TFA]) and then spotted on the target plate. Peptide spectra were acquired in positive reflection mode, averaging about 1000 laser shots per MALDI-TOF spectrum. Mass spectra were calibrated using the calibration mixtures CalMix1 and CalMix2 (Applied Biosystems). The mass spectra were analyzed using the Applied Biosystems Data Explorer® V 4.8 software and peptide mass lists were generated. Briefly: 1. advanced baseline correction setting peak width = 31, flexibility = 0.5, degree = 0.1; 2. noise filter setting noise removal std. dev. to remove = 2; 3. peak deisotoping; 4. calibration by importing a calibration spectrum. The peptide masse list was then analyzed using Microsoft Office Excel. A manually chosen subset of peptide masses were used to search against the NCBI non redundant database applying the peptide mass fingerprint algorithm on the Mascot web site (http://www.matrixscience.com). The search algorithm was set to allow carbamidomethylation on cystein residues, oxidation on methionin residues and a maximum of 1 missed cleavage. The peptide mass tolerance was set 0.2 Da. Proteins were considered as identified when the probability-based score was above the significance threshold ($p < 0.05$). Identification was confirmed by comparing the calculated molecular mass and isoelectric point values from the identified proteins with the observed values on the 2D gel.

C 9.4 Immunoblotting

C 9.4.1 Western Blotting

Western blotting was done using either the Mini Trans-Blot® system (BioRad) or the Multiphor™ II system (Amersham). Proteins were transferred to a nitrocellulose membrane (Protran BA85 0.45 µm, Schleicher and Schuell [Sambrook et al., 1989; Kyhse-Andersen 1984]).

C 9.4.2 Detection of immunogenic proteins

The blotting membrane was incubated with porcine sera. Preimmune sera were obtained from pigs before experimental infection and convalescent sera were obtained from pigs 21 day post experimental infection with *A. pleuropneumoniae*. Sera were diluted 1:100 in PBST (8 g NaCl, 0.2 g KH_2PO_4, 1.44 g Na_2HPO_4, 0.2 g KCl, 0.5 ml Tween 20, ad 1000 ml). As second antibody an alkaline phosphatase-conjugated goat anti swine IgG antibody was used in a 1:5,000 dilution. BCIP (5-bromo-4-chloro-3-indol phosphate) and NBT (nitro blue tetrazolium) were used as substrates for visualization of immunogenic proteins (Sambrook et al., 1989).

D Results

D 1 Phenotypic characterization of *A. pleuropneumoniae* Δ*arcA*

D 1.1 Construction of an *arcA* deletion mutant of *A. pleuropneumoniae*

The putative ArcA protein of *A. pleuropneumoniae* was identified by BLAST homology search using the *E. coli* anoxic redox control protein (gi:16132218) as a template. A highly homologous protein of *A. pleuropneumoniae* serotype 1 strain 4074 was found (gi:53729099) which is 80% identical to the *E. coli* ArcA. A protein entirely identical to the *A. pleuropneumoniae* serotype 1 ArcA homologue is encoded within the unfinished genome sequence of *A. pleuropneumoniae* serotype 7 (Fig. 3).

An isogenic *arc*A deletion mutant of *A. pleuropneumoniae* AP76 was constructed by allelic exchange of the wild type *arc*A gene with an in frame deletion lacking 186 bp at position 94 to 279 of the *arcA* ORF. The truncated ArcA protein lacks the phosphorylation site on Asp54. The resulting *A. pleuropneumoniae arcA* mutant was designated as *A. pleuropneumoniae* Δ*arcA* and verified using PCR, Southern blotting, and nucleotide sequence analysis; the absence of gross genomic rearrangements was confirmed by PFGE (Fig. 4). For complementation *A. pleuropneumoniae* Δ*arcA* was transformed with plasmid pARC1320 or pARC1340 and designated as *A. pleuropneumoniae* Δ*arcA* + pARC1320 or *A. pleuropneumoniae* Δ*arcA* + pARC1340.

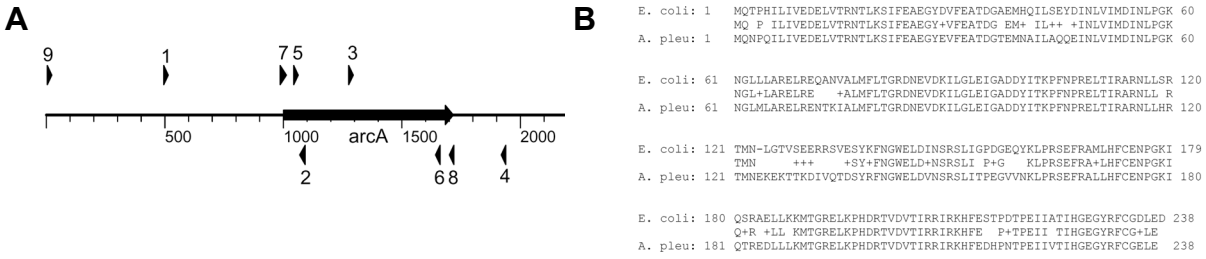


Fig. 3: A: Map of *A. pleuropneumoniae* contig 390. The *arcA* gene and primers used in the present study are shown. Primer binding sites are indicated as arrowheads.

B: Alignment of *E. coli* ArcA and *A. pleuropneumoniae* homologue. Alignments were made using the Blast 2 Sequences program at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov, Tatusova and Madden 1999). Both proteins are 88% similar and 80% identical.

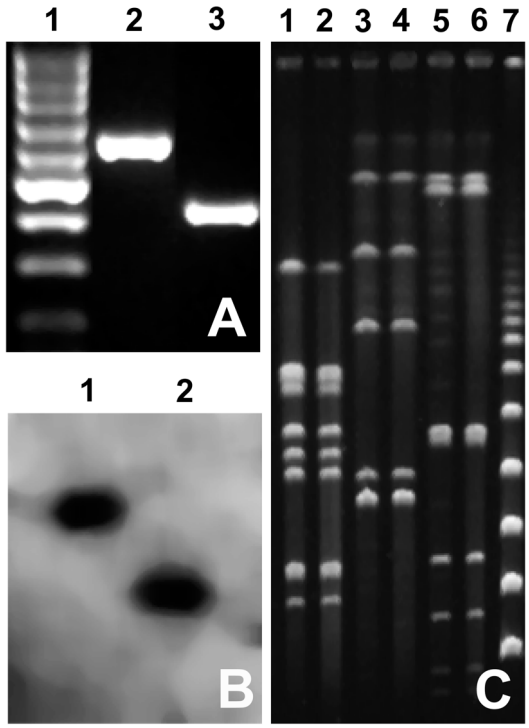


Fig. 4: Verification of *arcA* deletion by PCR, Southern blot analysis, and PFGE. A; Agarose gel electrophoresis of a DNA marker (100 bp ladder (NEB, lane 1) and PCR products obtained with primers oArcA5 and oArcA6 from genomic *A. pleuropneumoniae* DNA. PCR products obtained from *A. pleuropneumoniae* wt (lane 2) and *A. pleuropneumoniae* Δ*arcA* (lane 3) were 618 bp and 432 bp respectively. B; Southern blot analysis of genomic DNA restricted with HaeIII and XmaI. Digestion of *A. pleuropneumoniae* wt DNA resulted in a 915 bp fragment (lane 1), and a 729 bp fragment was obtained for *A. pleuropneumoniae* Δ*arcA* (lane 2). C; PFGE analysis to exclude genomic rearrangements. Genomic DNA of *A. pleuropneumoniae* wt (lanes 1, 3, 5) and *A. pleuropneumoniae* Δ*arcA* (lanes 2, 4, 6) was restricted with PspOMI (lanes 1, 2), AscI (lanes 3, 4) and NotI (lanes 5, 6). Phage lambda concanamers were loaded as a size marker (lane 7).

D 1.2 Virulence studies

Aerosol infection with both *A. pleuropneumoniae* wt (group 1) and *A. pleuropneumoniae* Δ*arcA* (group 2) led to an increase in body temperature above 40°C in all infected animals. However, animals infected with *A. pleuropneumoniae* Δ*arcA* showed a significantly lower clinical score ($p = 0.043$) than animals infected with *A. pleuropneumoniae* wt (Fig. 5A).

In order to investigate if the *arcA* deletion mutant could persist at least one week, four animals in group 2 were sacrificed seven days post infection. All remaining animals were sacrificed on day 21 post infection. At necropsy, three out of eight pigs in group 2 had no macroscopically visible lung lesions compared to one out of eight animals in group 1. The lung lesion score of group 2 was clearly lower than that of group 1 (Fig. 5B). Histological examination revealed no differences in the morphology of lung lesions in both groups.

Reisolation of the challenge strains from intact lung at necropsy 21 days post infection succeeded in all animals of group 1 but only in one out of four animals of group 2; at

necropsy on day 7 post infection reisolation succeeded in two out of four animals of group 2 (Table 2). The reisolation score of *A. pleuropneumoniae* Δ*arcA* was significantly reduced in comparison to the *A. pleuropneumoniae* wt group (Fig. 5C). From pneumonic lung the challenge strain could be reisolated in all seven animals with lesions in group 1 and in four out of five animals with lesions in group 2, with no difference observed between day 7 and day 21 post infection (Table 2).

Serum samples were obtained 1 week before and 3 weeks after experimental infection. Before infection, no *A. pleuropneumoniae* specific antibodies could be detected. On day 21 post challenge animals in both groups showed a strong humoral immune response, and no significant difference was observed (Table 2).

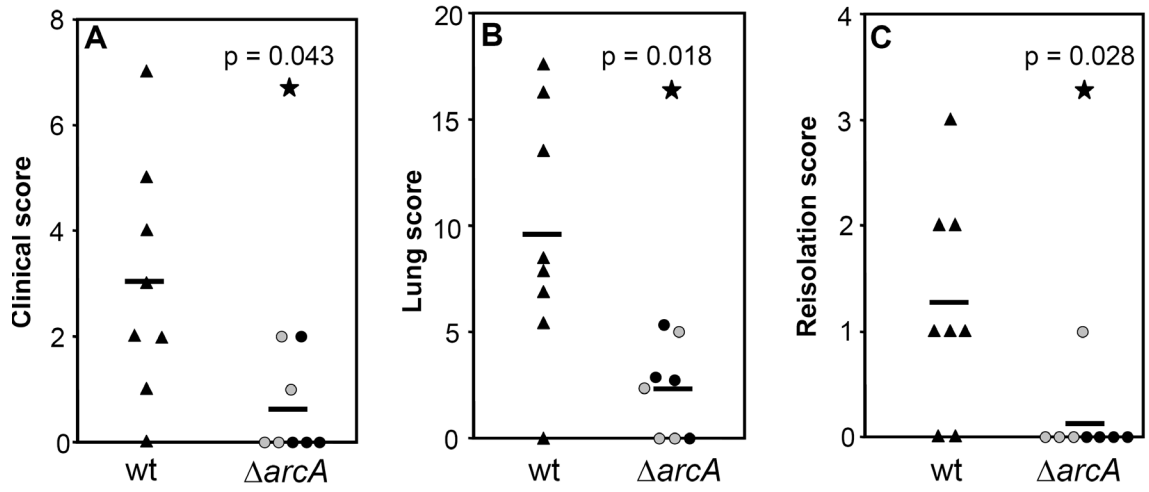


Fig. 5: Virulence of *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* compared in an aerosol infection model. Clinical score (A), lung lesion score (B) and reisolation score (C) of pigs infected with *A. pleuropneumoniae* wt (▲) or Δ*arcA* (○ [sacrificed on day 7]; ● [sacrificed on day 21]). Each triangle or circle represents one animal. The horizontal line represents the arithmetic mean. The asterisks denote statistical significance ($p \leq 0.05$) of differences between the entire *A. pleuropneumoniae* Δ*arcA* group (animals sacrificed at day 7 [four animals] and 21 post infection [four animals]) and *A. pleuropneumoniae* wt group (all sacrificed on day 21 post infection) as obtained using the Wilcoxon Signed-Rank Test. The animals sacrificed at different time points post infection were considered as a single group since both lung lesion and reisolation scores do not increase after day 7 of an *A. pleuropneumoniae* infection. Therefore, the approach might have resulted at the most in an underestimation of differences between the two groups.

Table 2: Virulence of *A. pleuropneumoniae* parent and Δ*arcA* mutant strain following aerosol challenge.

Challenge strain	No. of animals	Challenge dose (OD_{600} [aerosolized] per 4 pigs)[a]	Necropsy time (day)	Serological response to:		No. of animals with reisolation of *A. pleuropneumoniae* at post mortem analysis in:			
				Detergent wash[b]	ApxIIA[c]	Tonsil	Lymph node	Lung	
								Pneumonic	Intact
AP76 wt	8	0.47	21	3275 ± 2742	28.9±23.6	4/8	6/8	7/7	8/8
AP76 ΔarcA	4	0.42	7	–	–	4/4	2/4	2/2	2/4
AP76 ΔarcA	4		21	3000 ± 2477	60.5±25.7	1/4	2/4	2/3	1/4

[a] Bacteria were grown aerobically in the presence of Tween®80 (0.1%) to the respective OD_{600} and diluted 1:30,000 with sterile saline; 13 ml of this dilution were aerosolized in the aerosol chamber.

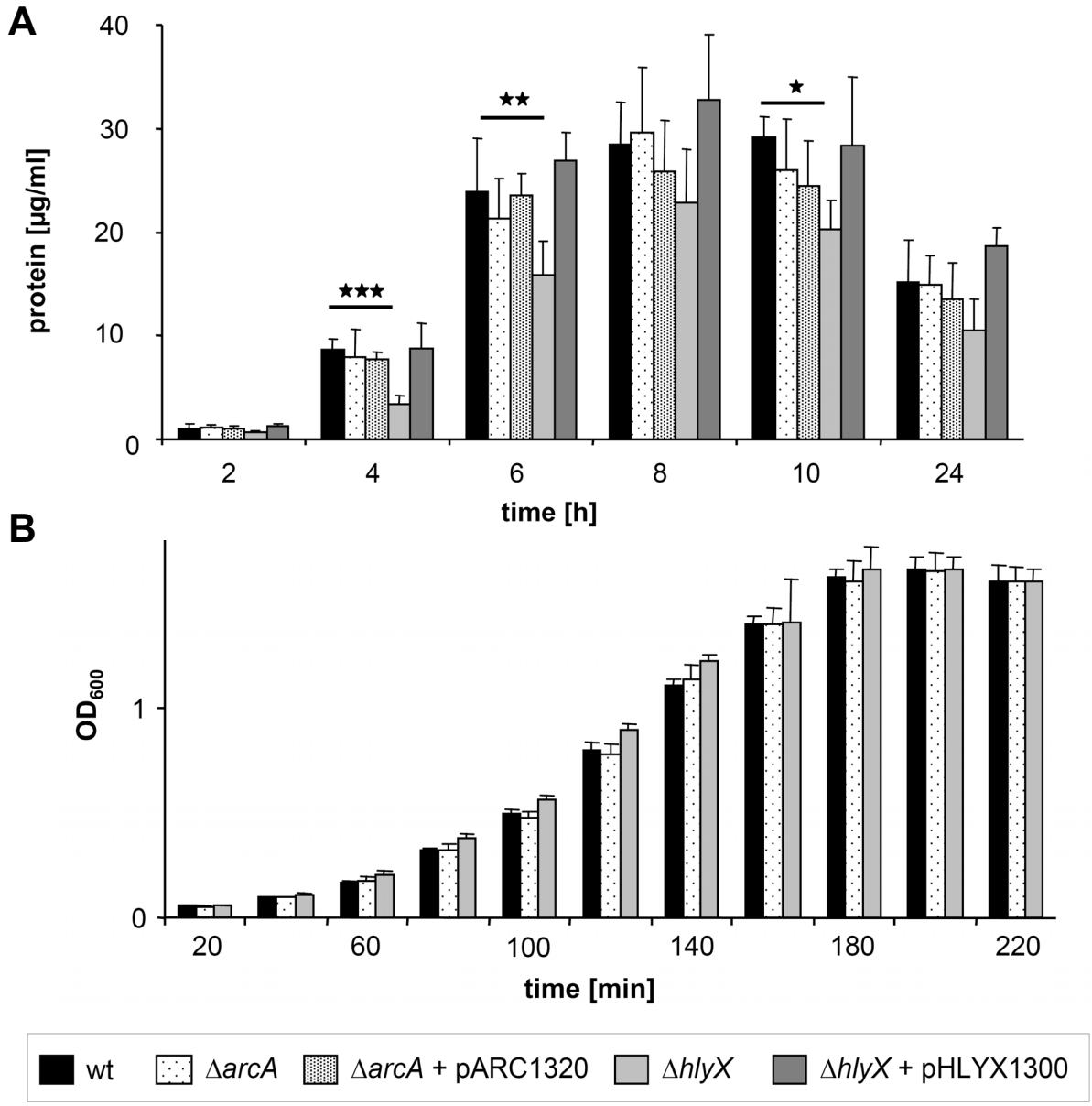
[b] The solid-phase antigen was prepared as described previously (Goethe et al., 2000); the number given is the arithmetic mean of the highest serum dilution resulting in an optical density twice as high as the negative control serum at a dilution of 1:100

[c] Recombinant ApxIIA protein was used as solid-phase antigen as described previously (Leiner et al., 1999) the number given is the arithmetic mean of the serum activity in ELISA units.

D 1.3 Growth and survival of *A. pleuropneumoniae* Δ*arcA*

Growth curves of *A. pleuropneumoniae* wt, *A. pleuropneumoniae* Δ*arcA* and the complemented strain *A. pleuropneumoniae* Δ*arcA* + pARC1320 were determined under anaerobic and aerobic conditions. For comparison anaerobic and aerobic growth of *A. pleuropneumoniae* Δ*hlyX*, and *A. pleuropneumoniae* Δ*hlyX* + pHLYX1300 (Baltes et al., 2005) were analyzed in parallel. The growth rates between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* exhibited no significant differences over a 24 h incubation period whereas *A. pleuropneumoniae* Δ*hlyX* showed a significantly reduced growth. This growth deficit was abrogated in the complemented strain *A. pleuropneumoniae* Δ*hlyX* + pHLYX1300. Complementation of the *arcA* gene resulted in a growth behaviour under anaerobic condition indistinguishable form *A. pleuropneumoniae* wt or *A. pleuropneumoniae* Δ*arcA*. Approximately two hours after inoculation all strains reached the exponential phase of growth which ended at 6 to 8 hours post inoculation. After reaching stationary phase (8 to 10 hours post inoculation) the protein content of the bacterial pellet is decreased at 24 hours after inoculation (Fig. 6A). Under aerobic conditions no growth difference could be observed between *A. pleuropneumoniae* wt, *A. pleuropneumoniae* Δ*arcA* and *A. pleuropneumoniae* Δ*hlyX* (Fig. 6B).

Survival kinetics of *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* in an anaerobic liquid culture under nutrient starvation are indistinguishable. Sixty hours post inoculation no culturable *A. pleuropneumoniae* wt or *A. pleuropneumoniae* Δ*arcA* were detectable. In contrast, A. *pleuropneumoniae* Δ*hlyX* showed a significantly increased survival, and aging was significantly reduced (Fig. 6C).



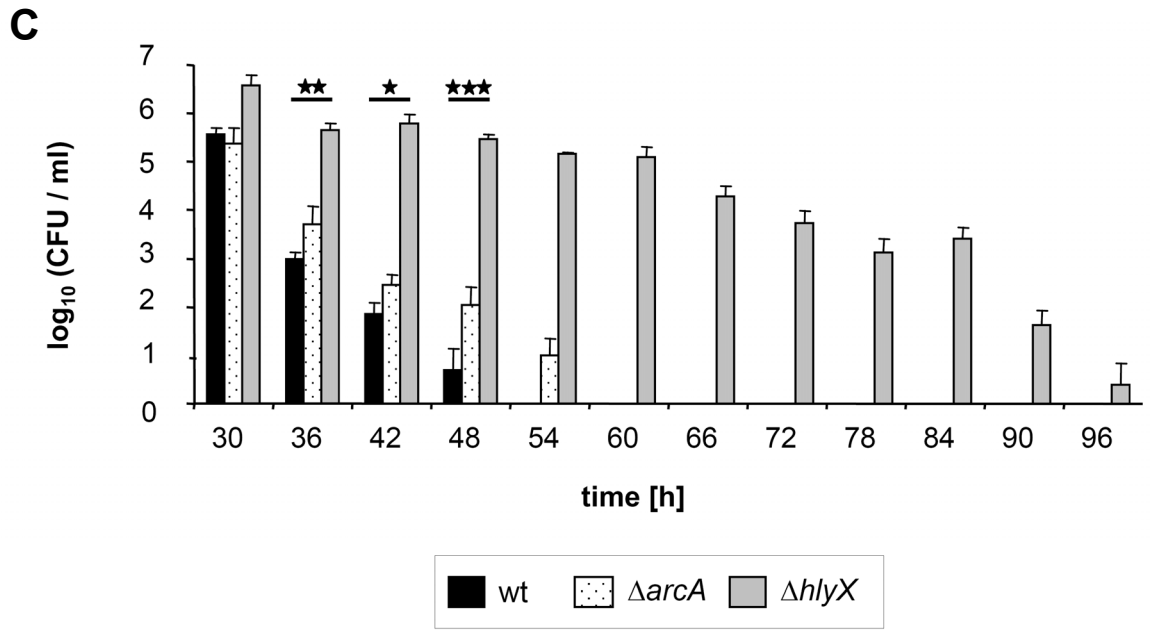


Fig. 6: Analysis of anaerobic growth (A), aerobic growth (B) and survival under anaerobic conditions accompanied by starvation (C) of *A. pleuropneumoniae* wt, Δ*arcA,* and Δ*hlyX*. Each experiment was repeated at least three times. Error bars represent the standard deviation. Asterisks denote statistical significance (one asterisk $p \leq 0.05$; two asterisks $p \leq 0.01$; three asterisks $p \leq 0.001$) of differences compared to *A. pleuropneumoniae* wt. Statistical analysis was performed using the Student's T-test.

D 1.4 Effect of *arcA* deletion on bacterial autoaggregation and biofilm formation

In liquid culture under aerobic or microaerophilic conditions both *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* grew as homogenous suspension (data not shown). Upon growth under anaerobic conditions *A. pleuropneumoniae* wt showed heavy autoaggregation. This phenotype was completely abolished by deletion of the *arcA* gene, and transformation of *A. pleuropneumoniae* Δ*arcA* with plasmid pARC1340 restored the autoaggregating phenotype (Fig. 7A).

Raster electron microscopy of anaerobically grown cultures showed that *A. pleuropneumoniae* wt was embedded in a threadlike extracellular matrix. In *A. pleuropneumoniae* Δ*arcA* an exogenous threadlike compound could also be observed. However, it seemed not to be attached to the bacterial surface, and threads had a relaxed structure. Complementation of *A. pleuropneumoniae* Δ*arcA* with plasmid pARC1340 resulted

in a phenotype which appeared to be associated even more tightly to the extracellular matrix than the wt strain (Fig. 7B).

As autoaggregation and the ability to form biofilm have been reported to be associated (Schembri et al., 2003), we investigated the ability of *A. pleuropneumoniae* Δ*arcA* to form biofilms. *A. pleuropneumoniae* wt attached to glass surfaces and formed biofilm. Six hours after inoculation no significant difference in biofilm formation on the bottom of the Erlenmeyer flask was observable between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA*. In A. *pleuropneumoniae* wt the biofilm further increased until 12 hours post inoculation (p. i.). Only at 18 p. i. a reduction of biofilm mass obtained by quantitative crystal violet staining was apparent. In *A. pleuropneumoniae* Δ*arcA,* however, the amount of biofilm was clearly reduced in comparison to the wildtype at 12 hours p. i., and the difference was statistically significant at 18 and 24 hours p.i. ($p = 0.013$ and 0.014, respectively, Fig. 7C). Complementation of *A. pleuropneumoniae* Δ*arcA* in trans with plasmid pARC1340 slightly overcompensated the defect in biofilm formation, which likely is due to the multiple copy number of *arcA* in the pARC1340 transformants; transformants containing the empty plasmid vector pLS88 behaved like the wild type (Fig. 7C). The lack of biofilm formation in *A. pleuropneumoniae* Δ*arcA* was not caused by differences in cell densities as obtained by determination of OD_{600} (data not shown).

A. pleuropneumoniae Δ*hlyX* was also tested for clumping and biofilm formation. Clumping of A. pleuropneumoniae in an anaerobic liquid culture appeared delayed at about 8 to 10 hours post inoculation. Biofilm formation of *A. pleuropneumoniae* Δ*hlyX* at an abiotic glass surface was comparable to *A. pleuropneumoniae* wt (data not shown).

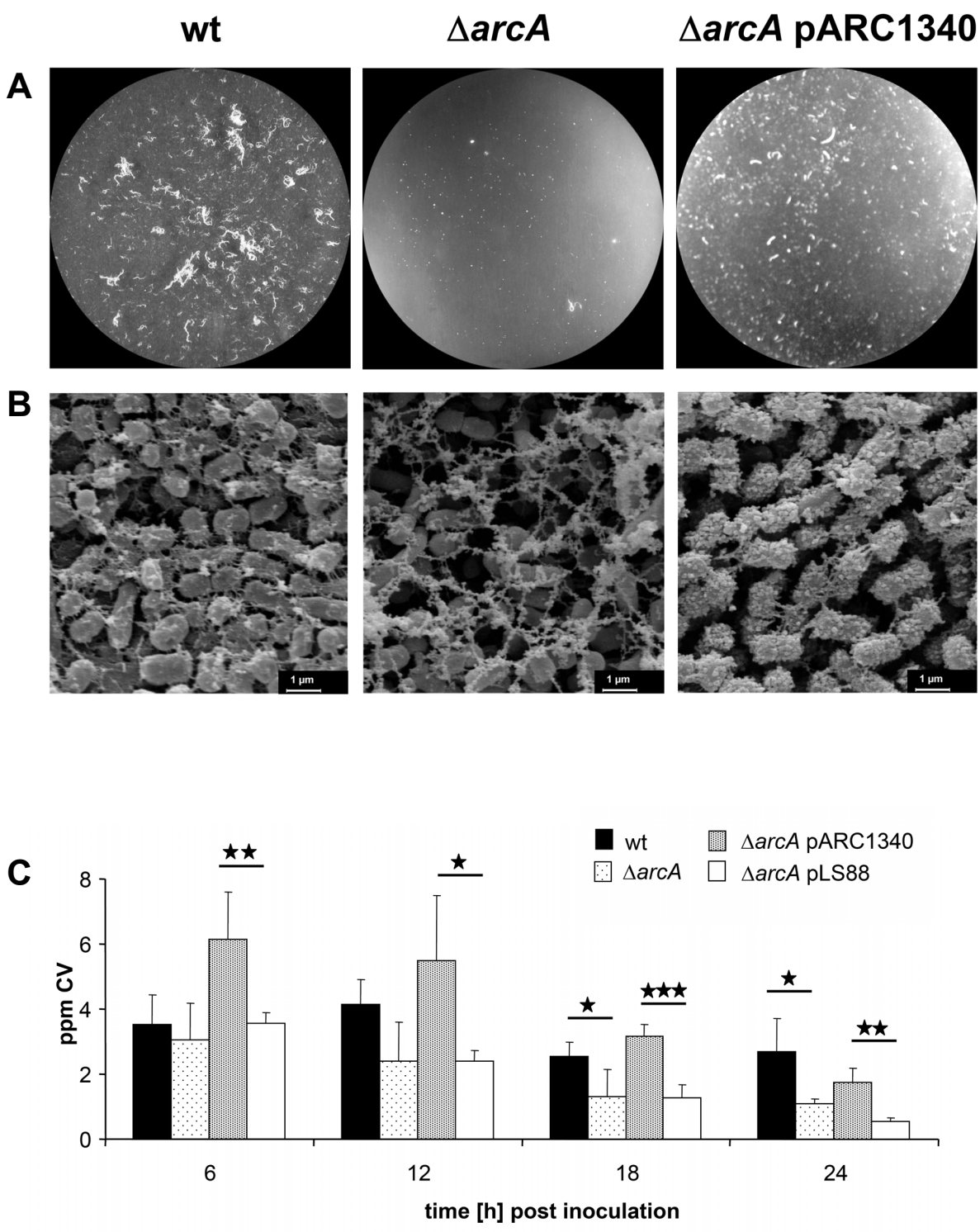


Fig. 7: Autoaggregation and biofilm formation. A: Autoaggregation of anaerobically grown cultures. B: Raster electron microscopy of cultures shown in A. C: Biofilm formation on the bottom of glass Erlenmeyer flasks. The biofilm was stained with crystal violet. Crystal violet bound to the biofilm was solubilised and measured at OD_{600}. Using a calibration curve the amount of crystal violet was calculated as ppm [w/v]. Error bars represent the standard deviation. Statistical analysis was performed using Student's T-test. Asterisks denote statistical significance ($p \leq 0.05$).

D 1.5 Effect of *arcA* deletion on malic enzyme activity

Initial proteomic analysis of ArcA regulated proteins revealed the malic enzyme (MaeB) as strongly downregulated by ArcA. An enzymatic assay for MaeB activity was employed (Geer et al., 1979) in order to confirm the proteomic results and to test the complementation in trans of *A. pleuropneumoniae* Δ*arcA*.

Whole cell lysates of anaerobically grown cultures of the *A. pleuropneumoniae* wt strain exhibited an average activity of 1.88 units of malic enzyme per gram protein, whereas the average activity of malic enzyme in *A. pleuropneumoniae* ∆*arcA* was determined as 11.08 units per gram protein (a 6-fold increase compared to the parental strain *A. pleuropneumoniae* wt). This upregulation of malic enzyme activity by deletion of *arcA* was statistically significant ($p = 0.001$). Complementation of *A. pleuropneumoniae* ∆*arcA* in trans with plasmid pARC1320 carrying the *arcA* gene and 977 bp upstream sequence resulted in a reduction of malic enzyme activity to wt-level (Fig. 8A). Deletion of the *hlyX* gene (Baltes et al., 2005) had no impact on malic enzyme activity (Fig. 8A).

When the bacteria where grown under aerobic conditions the malic enzyme activity was between 20 und 25 units per gram protein with no significant differences between *A. pleuropneumoniae* wt, *A. pleuropneumoniae* ∆*arcA* or *A. pleuropneumoniae* ∆*hlyX* (Fig. 8B).

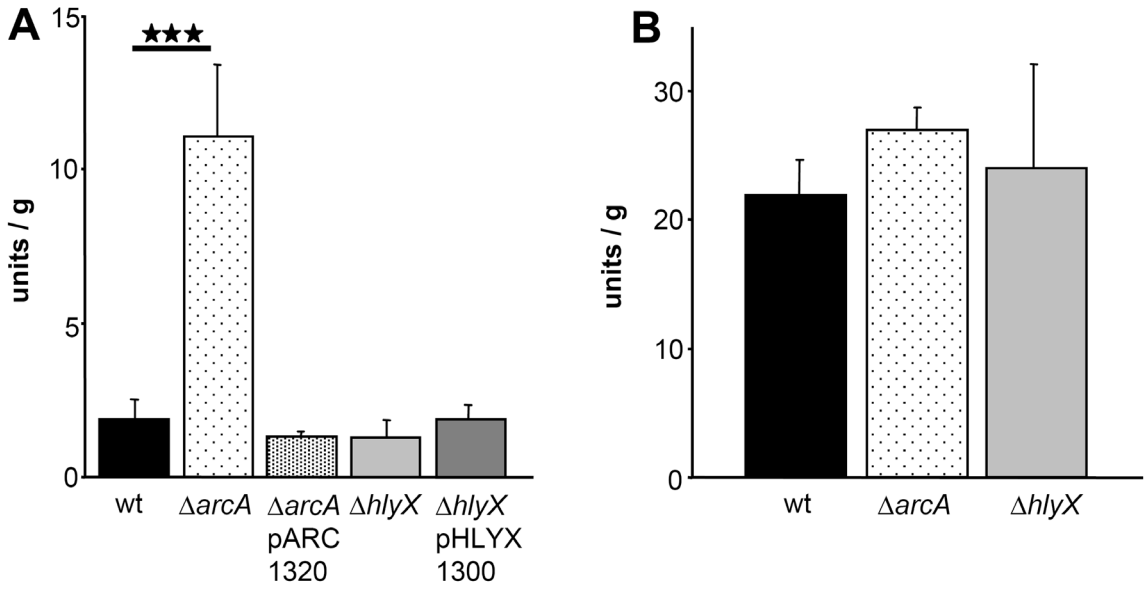


Fig. 8: Complementation of *A. pleuropneumoniae* Δ*arcA* in trans restored wt level of malic enzyme activity. *A. pleuropneumoniae* was grown either anaerobically (A) or aerobically (B), and malic enzyme activity was obtained using colorimetric measurement of NADPH formation from $NADP^+$ when L-malate is oxidatively decarboxylated to pyruvate in whole cell lysates. Malic enzyme activity was normalized to total protein content. Each experiment was repeated at least three times. Error bars represent the standard deviation. Asterisks denote statistical significance ($p \leq 0.05$) of differences compared to *A. pleuropneumoniae* wt as obtained by Student's T-test.

D 2 The ArcA regulon of *A. pleuropneumoniae*

D 2.1 Global transcription analysis of *A. pleuropneumoniae* Δ*arcA*

In order to identify the regulon of the ArcAB two component signal transduction system of *A. pleuropneumoniae* a global expression profiling was performed using a DNA microarray analysis. The array chip used was designed for *A. pleuropneumoniae* serotype 5b strain L20 (Deslandes et al., 2007). However, cDNA obtained from *A. pleuropneumoniae* serotype 7 isolate 76 (*A. pleuropneumoniae* wt) transcripts bound to the vast majority of the array spots (Fig. 9A). Six independent expression profiles each of the anaerobically grown parental strain *A. pleuropneumoniae* wt and the *arcA* deletion mutant *A. pleuropneumoniae* Δ*arcA* were analysed. The gene expression ratios between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* were reproducible for the different biological repeats (Fig. 9B). Of the 2025 protein coding genes of *A. pleuropneumoniae* 5b L20 that are presented on the array chip the expression of 16 genes was increased greater then 2-fold and expression of a further 77 genes was increased by a factor between 1.5 and 2 in *A. pleuropneumoniae* wt compared to the *arcA* deletion mutant. These genes are either directly or indirectly upregulated by ArcA in the parental strain. In *A. pleuropneumoniae* Δ*arcA* the expression of 42 genes was increased greater then 2-fold and 64 genes were increased by a factor between 1.5 and 2 compared to the parental strain *A. pleuropneumoniae* wt. The expression of these genes was downregulated in *A. pleuropneumoniae* wt by ArcA (Fig. 10; Appendix G 1 and G 2).

A B

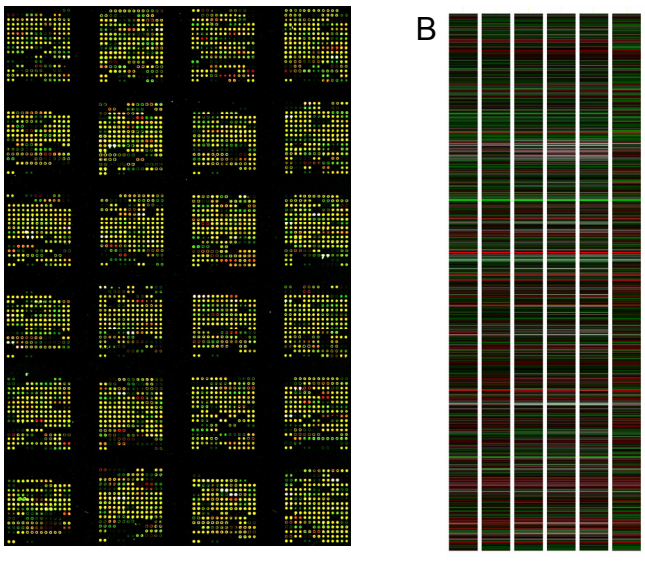


Fig. 9: Microarray analysis of anaerobically grown *A. pleuropneumoniae* wt in comparison to *A. pleuropneumoniae* Δ*arcA*. A: Readout of a microarray slide hybridized with Cy5 labelled cDNA of *A. pleuropneumoniae* wt and Cy3 labelled cDNA of *A. pleuropneumoniae* Δ*arcA*.
B: Labelling reactions were repeated six times. Each row represents all ORFs of the *A. pleuropneumoniae* genome as horizontal lines.

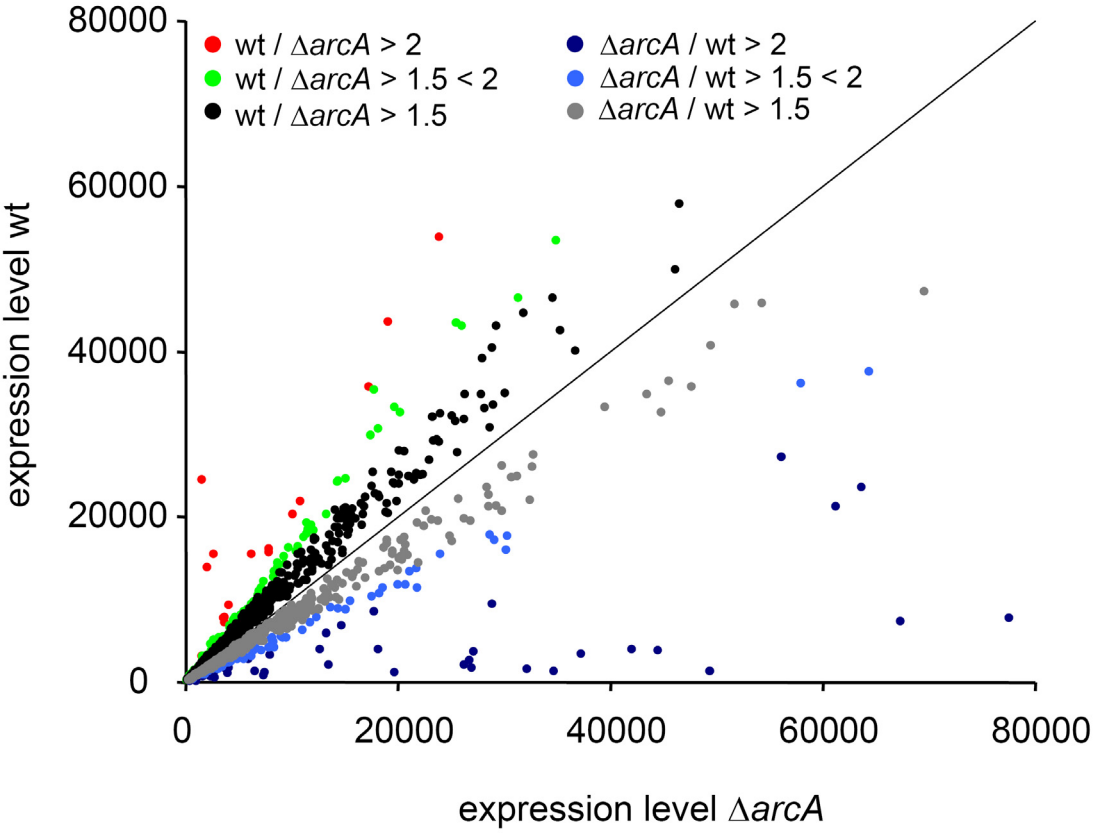


Fig. 10: Summary of microarray results wt vs. Δ*arcA*. Each spot represents a gene that has been identified to be significantly affected by ArcA ($p \leq 0.05$). Spots appearing above the diagonal line represent genes that were upregulated by ArcA; those that appear below exhibited a higher expression level in the *arcA* deletion mutant caused by a downregulation due to ArcA in *A. pleuropneumoniae* wt. Green spots were upregulated significantly due to ArcA by more then 1.5-fold and red spots were upregulated by more then 2-fold. Light blue spots were downregulated significantly by more then 1.5-fold and dark blue spots were downregulated by more then 2-fold due to ArcA.

D 2.1.1 Functional classification of ArcA regulated genes

A functional classification of the genes that were identified to be regulated by ArcA was performed according to the database of “Cluster of Orthologous Groups of proteins” (COGs) (http://www.ncbi.nlm.nih.gov/COG/; Tatusov et al., 1997). ArcA controlled the expression of genes of almost all prokaryotic COGs. In some functional categories differences in the number of upregulated and downregulated genes by ArcA were obvious. In the category “carbohydrate transport and metabolism (G)” seven genes were downregulated by ArcA and none upregulated. Twenty-one genes belonging to the category “energy production and

conversion (C)" were downregulated by ArcA compared to six genes that were upregulated. Similarly, more then twice the number of genes in the category "inorganic ion transport and metabolism (P)" were downregulated by ArcA than upregulated. In the categories "replication, recombination and repair, transcription (L)" and "cell cycle control, cell division, chromosome partitioning (D)", however, considerably more genes were upregulated than downregulated by ArcA (Fig. 11).

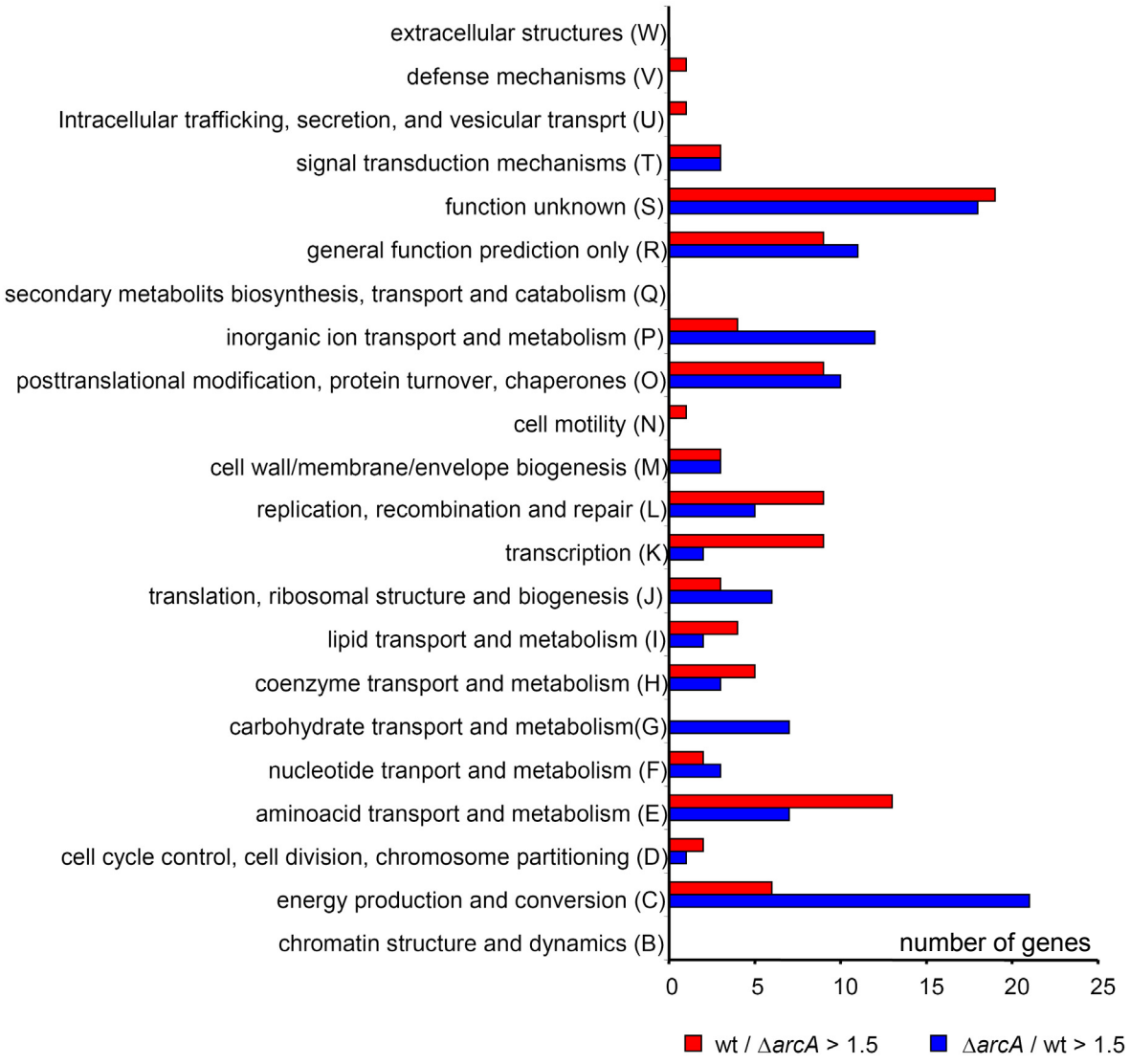


Fig. 11: Functional classification of ArcA regulated genes according to the database Cluster of Orthologous Groups of Proteins (COGs). The classification was adopted from the "Simple Yet Powerful Genome Browser" database for *A. pleuropneumoniae* Ser 5 L20 (http://informatics.bio.nrc.ca/ap5b)

D 2.1.2 Analysis of the ArcA regulon

The four genes upregulated strongest by ArcA (*ape0761, ape0760, ape0758* and *serC*) are located side by side on the genome of *A. pleuropneumoniae* and exhibited a decline in upregulation; they belong to a putatively ArcA regulated gene cluster or operon, and three of the genes are homologues to *E. coli* DNA modification enzymes. Putative protein Ape0761 exhibits homology to the methylation subunit of *E. coli* restriction endonuclease EcoP15I, which belongs to a type III restriction-modification system. Ape0760 is a putative DEAD/DEAH box helicase domain protein with homology to type I restriction-modification systems. Ape0758 is homologue to an *E. coli* DNA/RNA helicase of the SNF2 family containing a DEAD/DEAH box helicase motif. Two further genes, *ape0309* showing homology to the restriction subunit of an *E. coli* type I restriction modification system and related helicases and gene *ape0841*, a putative ATP-dependent helicase, were slightly increased in *A. pleuropneumoniae* wt.

Enzymes of the respiratory chain catalyzing the oxidation of high-energy substrates did not show a unanimous regulation by ArcA in *A. pleuropneumoniae*. Thus, the genes *glpA*, *glpB* and *glpC*, encoding for the anaerobic glycerol-3-phosphate dehydrogenase operon, were upregulated in the wt strain compared to the *arcA* deletion mutant. The expression of the anaerobic glycerol-3-phosphate repressor gene (*glpR*) was simultaneously decreased by ArcA.

The expression of a putative NADPH oxidoreductase gene of *A. pleuropneumoniae* exhibiting homology to *mdaB* of *E. coli* which catalyzes the reduction of quinones by oxidation of NADPH was found to be increased by ArcA.

However, the formate dehydrogenase was identified to be downregulated by ArcA in *A. pleuropneumoniae* wt. Six open reading frames (*fdhD*, *fdxG* [APL_0892], *fdxG* [APL_0893], *fdxH*, *fdnI*, *fdhG*) with homology to genes encoding for *E. coli* formate dehydrogenases were identified in *A. pleuropneumoniae*; they were strongly repressed by ArcA under anaerobic conditions of growth.

The expression of genes encoding for either flavin mononucleotide-dependent D-lactate dehydrogenase (*dld*) or L-lactate dehydrogenase (*lldD*) of the *A. pleuropneumoniae* respiratory chain were downregulated by ArcA. The putative malate:qinone oxidoreductase (*mqo*) was also repressed by ArcA as well as the *putA* gene, encoding for a bifunctional protein which includes proline dehydrogenase activity transferring electrons from proline into the respiratory chain. The genes *ykgF* encoding a putative electron transport protein and *ykgE* encoding a putative dehydrogenase subunit and *ape1960* encoding a protein homologue to a possible sulfite oxidase of *Mannheimia haemolytica* were also downregulated by ArcA.

Terminal reductases which transfer electrons of the respiratory chain to alternative electron acceptors other than oxygen under anaerobic conditions were also not regulated unanimously by ArcA. The operon encoding for the anaerobic dimethylsulfoxide reductase (*dmsABC*) was positively regulated by ArcA. However, all homologues to genes of the *E. coli* nitrite reductase compelex, *nrfABCD* were found to be negatively regulated. Likewise, five genes of *A. pleuropneumoniae* (*ccmA*, *ccmB*, *ccmC*, *ccmE*, *ccmF*) showing homology to the *E. coli* type 1 cytochrome c biogenesis system which is essential for functional NrfA and NrfB were downregulated. This was also observed to the gene *nrfE* which is essential for formate dependent nitrite reduction, the gene *napF* which is part of the periplasmic nitrate reductase operon of *E. coli,* and both genes encoding the cytochrome bd-I terminal oxidase (*cydA*, *cydB*) of *A. pleuropneumoniae* which catalyzes the transfer of electrons from ubiquinol to oxygen.

Proteins that facilitate membrane transport of certain compounds were also under control of ArcA in *A. pleuropneumoniae*. The expression of genes encoding transport-associated proteins for zinc (*znuC*, *znuA*) and serine (*sdaC*) were upregulated by ArcA. In contrast, transport systems for L-lactate (*lctP*) and sulfate (cysZ) were downregulated as were the sodium/glutamate (*gltS*) and formate/nitrate (*yrhG*) symporter as well as oxalate/formate (ape0969) and sodium/proton (*nhaA*) antiporter. Also, expression of the genes *ape1350* and *ape1353* encoding homologues to di- and tricarboxylate transporters of *Haemophilus influenzae*, a glutathione-regulated potassium-efflux system protein encoded by *kefAB*, the gene *ape0961* encoding a homologue to a possible malonate efflux carrier of *Mannheimia haemolytica*, and the gene *ulaA* encoding the predicted ascorbate transporter were downregulated by ArcA.

Iron uptake was also controlled by ArcA of *A. pleuropneumoniae*. The expression of *afuA_2*, which is homologous to a putative periplasmic-iron-binding protein of *E. coli* and two adjacent ORFs (*ape2007* and *ape 2008*) encoding homologues to *E. coli* outer membrane receptor proteins, mostly iron transport, were upregulated by ArcA. Two genes with homology to a putative permease component for ferric iron (*afuB*) and a putative periplasmic-iron-binding protein (*afuA*) of *E. coli*, and *frpB* a putative iron regulated outer membrane protein were downregulated by ArcA.

The genes encoding several enzymes with functions in the glycolysis, gluconeogenesis and citric acid cycle were affected by ArcA in *A. pleuropneumoniae*. Two committed steps of the gluconeogenesis were concerned. The genes *pck* encoding the phosphoenolpyruvate carboxykinase and *fbp* encoding for fructose-1,6-bisphosphatase were downregulated by ArcA as were the last steps of glycolysis. Thus, expression of all genes encoding for components of the pyruvate dehydrogenase complex (*lpdA*, *aceF*, *aceE*) were downregulated by ArcA. LpdA is also part of the 2-oxoglutarate dehydrogenase complex in

the citric acid cycle. The genes *lpdA*, *aceF* and *aceE* are likely to be transcribed as an operon. Malate can be converted to pyruvate by the NADP-dependent malic enzyme. The respective gene *maeB* was also downregulated by ArcA as was the already mentioned malate:quinone oxidoreductase (*mqo*) gene, whose gene product is part of the respiratory chain as well as of the citric acid cycle.

The gene *sucD* encoding for the alpha subunit of the succinyl-CoA synthetase of *A. pleuropneumoniae* was identified to be upregulated by ArcA as were the gene *accD* encoding the acetyl-CoA carboxyltransferase which is one of the key enzymes for fatty acid synthesis and the genes *fabA* and *fabZ* encoding for isoenzymes that are necessary for the synthesis of unsaturated fatty acids.

Several genes involved in serine metabolism of *A. pleuropneumoniae* were identified to be upregulated by ArcA. Thus, two out of three enzymes of the serine biosynthesis pathway, the 3-phosphoserine aminotransferase and the phosphoserine phosphatase encoded by the genes *serC* and *serB* were upregulated by ArcA. The reaction catalyzed by SerC is under physiological conditions reversible whereas the SerB-catalyzed reaction prefers the formation of L-serine. Additionally, the gene for the L-serine transport protein, *sdaC* was identified to be upregulated by ArcA as were the genes encoding for enzymes that use L-serine as a substrate. These were the serine acetyltransferase encoded by *cysE* catalyzing the first step of cysteine synthesis, the phosphatidylserine synthase (*pssA*) that is necessary for the formation of the phospholipid phosphatidylserine, and the L-serine dehydratase, catalyzing the irreversible transformation of serine into pyruvate and ammonia. The 3-phosphoserine aminotransferase encoded by *serC* is also part of the vitamin B_6 biosynthesis pathway in *E. coli*. Two genes that were identified to be also upregulated by ArcA, *pdxT* and *pdxS*, encode for the synthetase and glutaminase subunits of a glutamine amidotransferase that has been described for *Bacillus subtilis* (Belitsky 2004) to be essential for pyridoxal 5´-phosphate biosynthesis which is the active form of vitamin B_6.

The global regulator ArcA not only increased its own expression but also effected the expression of several proteins involved in regulatory processes in bacterial cells. Expression of *cyaA* encoding the adenylate cyclase and *icc* encoding a 3´,5´-cyclic-nucleotide phosphodiesterase was repressed by ArcA of *A. pleuropneumoniae*. Thereby, synthesis of the second messenger cAMP was affected by ArcA. The expression of *gntR* was upregulated by ArcA. GntR, is a repressor of the Entner-Doudoroff pathway and the GntI system which allows the entry of glyconate into the central glycolytic metabolism (Conway 1992). The expression of the gene encoding the sensor protein NarQ was upregulated by ArcA of *A. pleuropneumoniae* contrary to *E. coli* where it has been reported that ArcA is not required for anaerobic induction of *narQ* (Darwin and Stewart 1995). The main signal for the NarQ sensor kinase of *E. coli* is nitrite. This signal is then transferred to the response

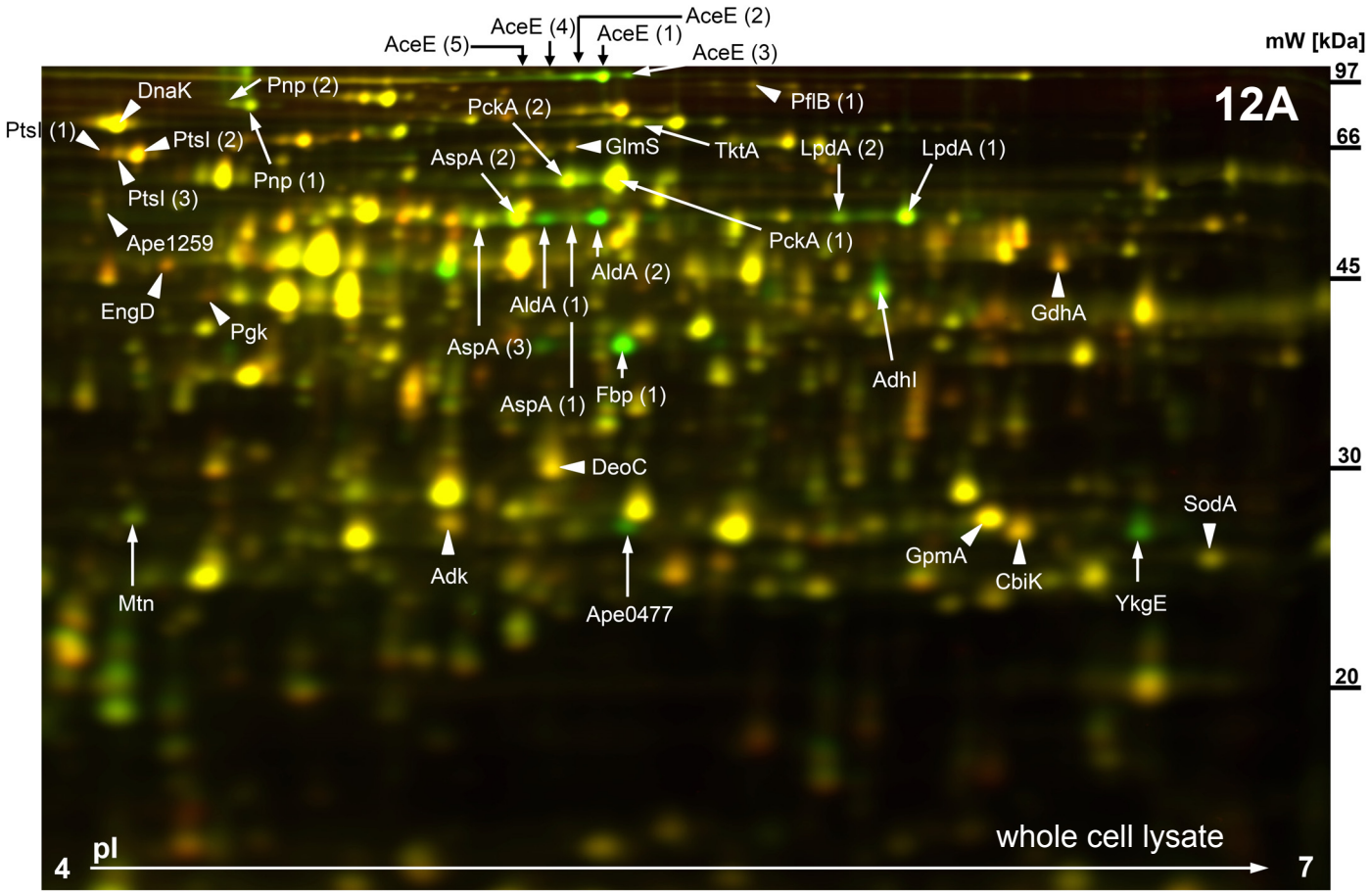
regulators NarL as well as NarP (Rabin and Stewart 1993) that adapt the metabolism onto nitrite or nitrate respiration. Although *narQ* expression was induced by ArcA anaerobically the general metabolism was not adapted to nitrite or nitrate respiration. The gene *cpxR* encoding a transcriptional regulator activating the transcription of stress-combative genes was identified to be upregulated in *A. pleuropneumoniae* as was the expression of the gene *asnC*. AsnC controls the expression of the aspartate-ammonia ligase gene and expression of the gene *ape0752* encoding for a putative HTH-type transcriptional regulator.

All chaperone proteins of the ArcA regulon were identified to be upregulated by ArcA of *A. pleuropneumoniae*. The expression of *hptG* was induced as was the expression of both genes encoding the GroEL-GroES complex, the expression of the gene encoding for the chaperone ClpB, and expression of two genes, *dnaK* and *dnaJ,* which are part of the DnaK system.

A cluster of genes (*rpsJ*, *rplC*, *rplD* and *rplW*) encoding for ribosomal proteins was slightly downregulated by ArcA.

D 2.2 Global protein expression analysis of *A. pleuropneumoniae* Δ*arcA*

The global transcription analysis aiming for the identification of differences between gene expression of *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* by DNA microarray approach was complemented with the identification of differences in protein pattern using two-dimensional difference gel electrophoresis (2D DIGE) and subsequent mass spectrometry. Different preparation methods of *A. pleuropneumoniae* proteins were performed in order to increase the resolution of proteins for comparison between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA*. 2D DIGE analyses were performed from i) - iii) whole cell lysates (Fig. 12A, 12B, 12C), iv) inner and outer membrane-associated proteins (Fig. 12D), v) outer membrane-associated proteins (Fig. 12E), vi) secreted proteins (Fig. 12F). Outer membrane proteins were analysed by quantitative PAGE (Fig. 13). Altogether 177 protein spots significantly increased in *A. pleuropneumoniae* wt compared to *A. pleuropneumoniae* Δ*arcA* were identified by 2D DIGE. Mass spectrometry of 58 of these spots yielded 31 different proteins (Table 3, 4). 193 spots were found to be significantly downregulated by ArcA in *A. pleuropneumoniae* and mass spectrometry of 74 of these spots resulted in 28 different proteins (Table 3, 5). Additionally to these 132 mass spectrometry analyses 28 protein spots belonging to non-significant but otherwise interesting protein spots on 2D gels were analysed by mass spectrometry (Table 6). Of the 160 MS analyses performed in total, 116 were performed by Q-TOF MSMS (Appendix G 6.1) and 44 by MALDI-TOF MS (Appendix G 6.2).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66
45
30
20
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AceE (2)
AceE (3)
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PflB (1)
PtsI (1)
PtsI (2)
PckA (2)
GlmS
TktA
LpdA (2)
LpdA (1)
AspA (2)
Pnp (1)
PtsI (3)
PckA (1)
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AldA (2)
EngD
Pgk
AldA (1)
GdhA
AspA (3)
AdhI
AspA (1)
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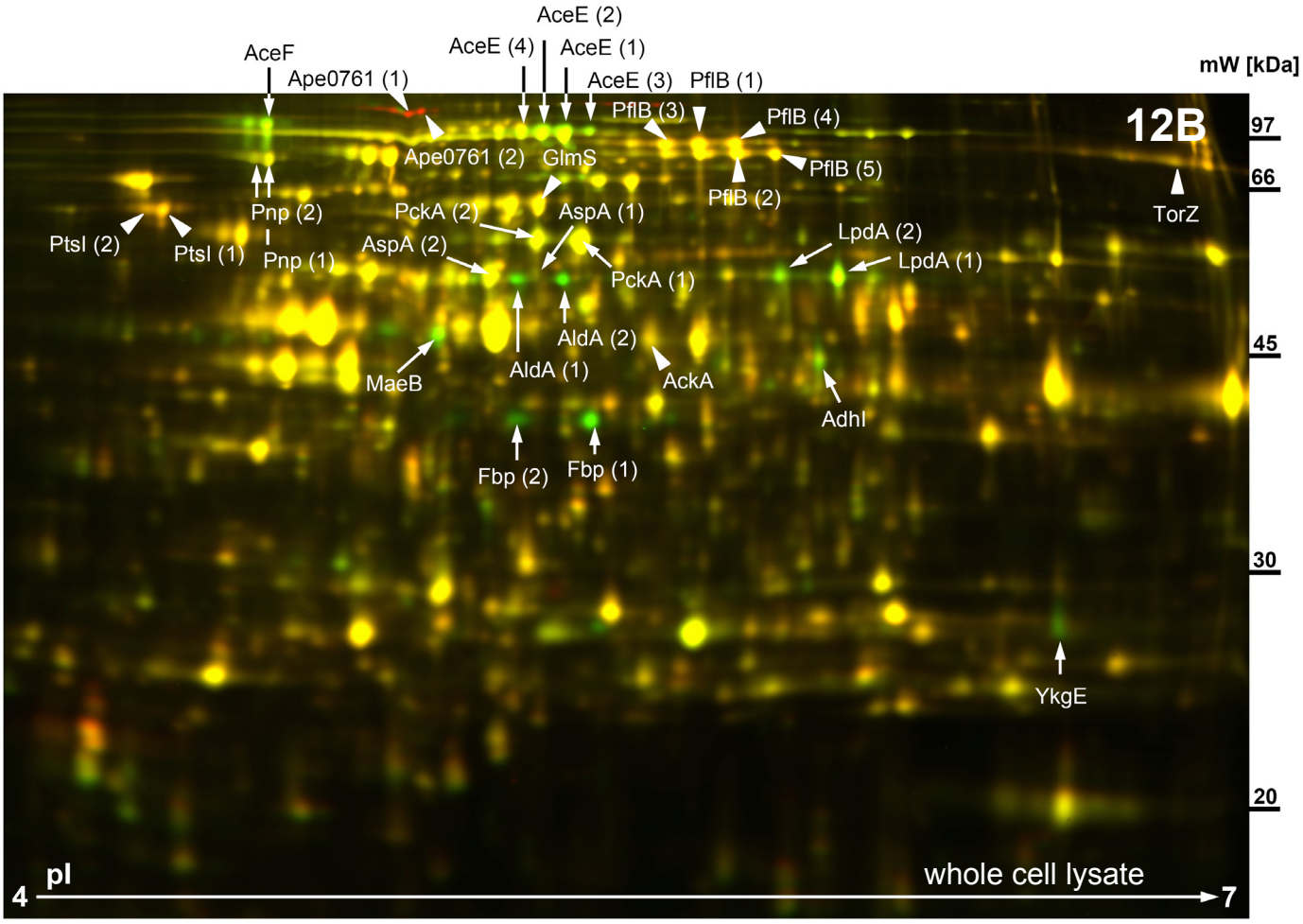
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PflB (3)
PflB (4)
Ape0761 (2)
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PflB (5)
PflB (2)
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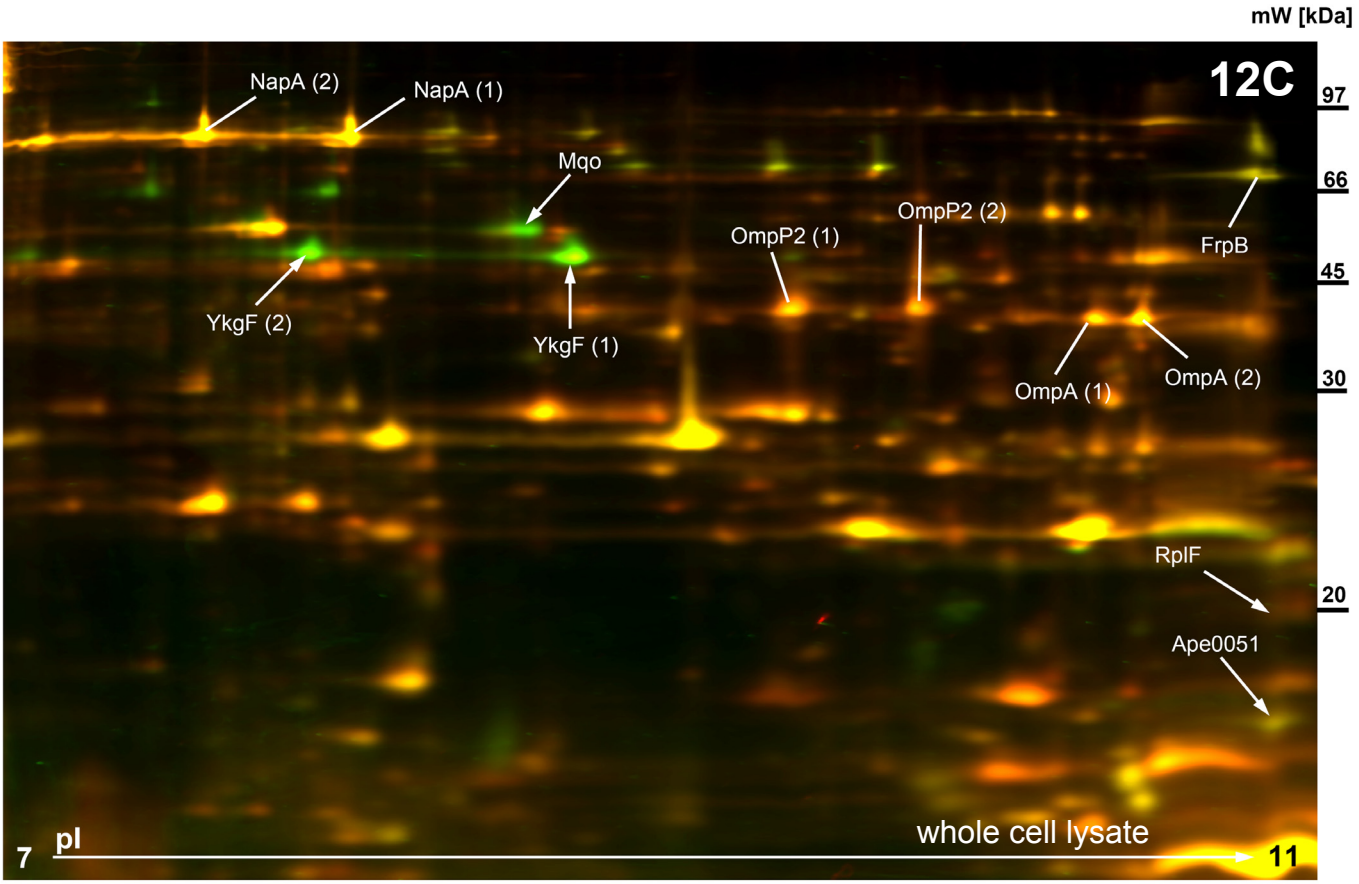
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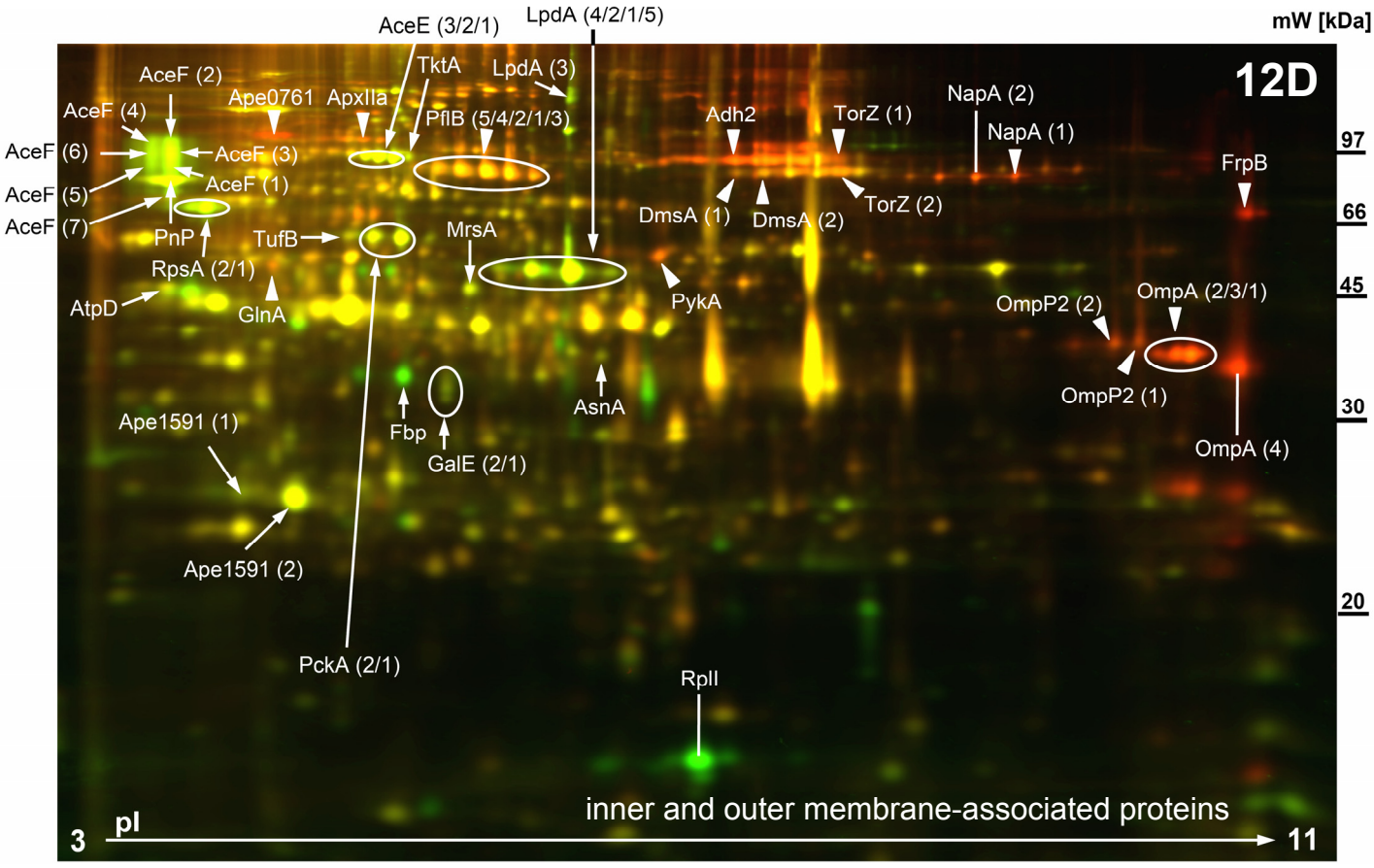
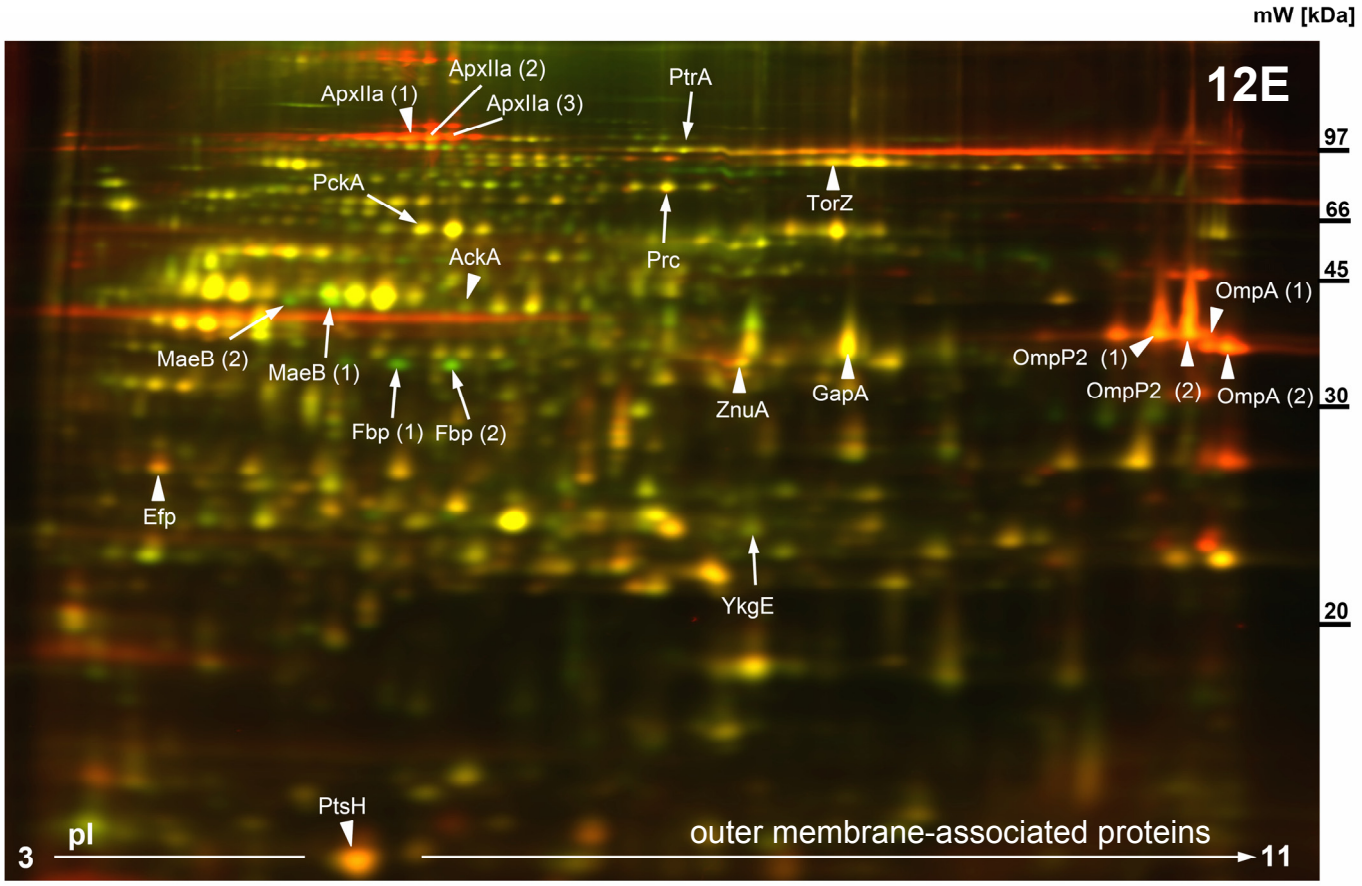
7
pI
11
whole cell lysate


mW [kDa]
12D
AceE (3/2/1)
LpdA (4/2/1/5)
AceF (2)
TktA
LpdA (3)
AceF (4)
Ape0761
ApxIIa
PflB (5/4/2/1/3)
Adh2
TorZ (1)
NapA (2)
NapA (1)
AceF (6)
AceF (3)
AceF (1)
FrpB
AceF (5)
AceF (7)
PnP
TufB
MrsA
DmsA (1)
DmsA (2)
TorZ (2)
RpsA (2/1)
AtpD
GlnA
PykA
OmpP2 (2)
OmpA (2/3/1)
Ape1591 (1)
Fbp
GalE (2/1)
AsnA
OmpP2 (1)
OmpA (4)
Ape1591 (2)
PckA (2/1)
RplI
97
66
45
30
20
3
pI
11
inner and outer membrane-associated proteins


mW [kDa]
12E
ApxIIa (2)
ApxIIa (1)
ApxIIa (3)
PtrA
PckA
TorZ
AckA
Prc
OmpA (1)
MaeB (2)
MaeB (1)
OmpP2 (1)
OmpP2 (2)
OmpA (2)
Fbp (1)
Fbp (2)
ZnuA
GapA
Efp
YkgE
PtsH
3
pI
outer membrane-associated proteins
11
97
66
45
30
20

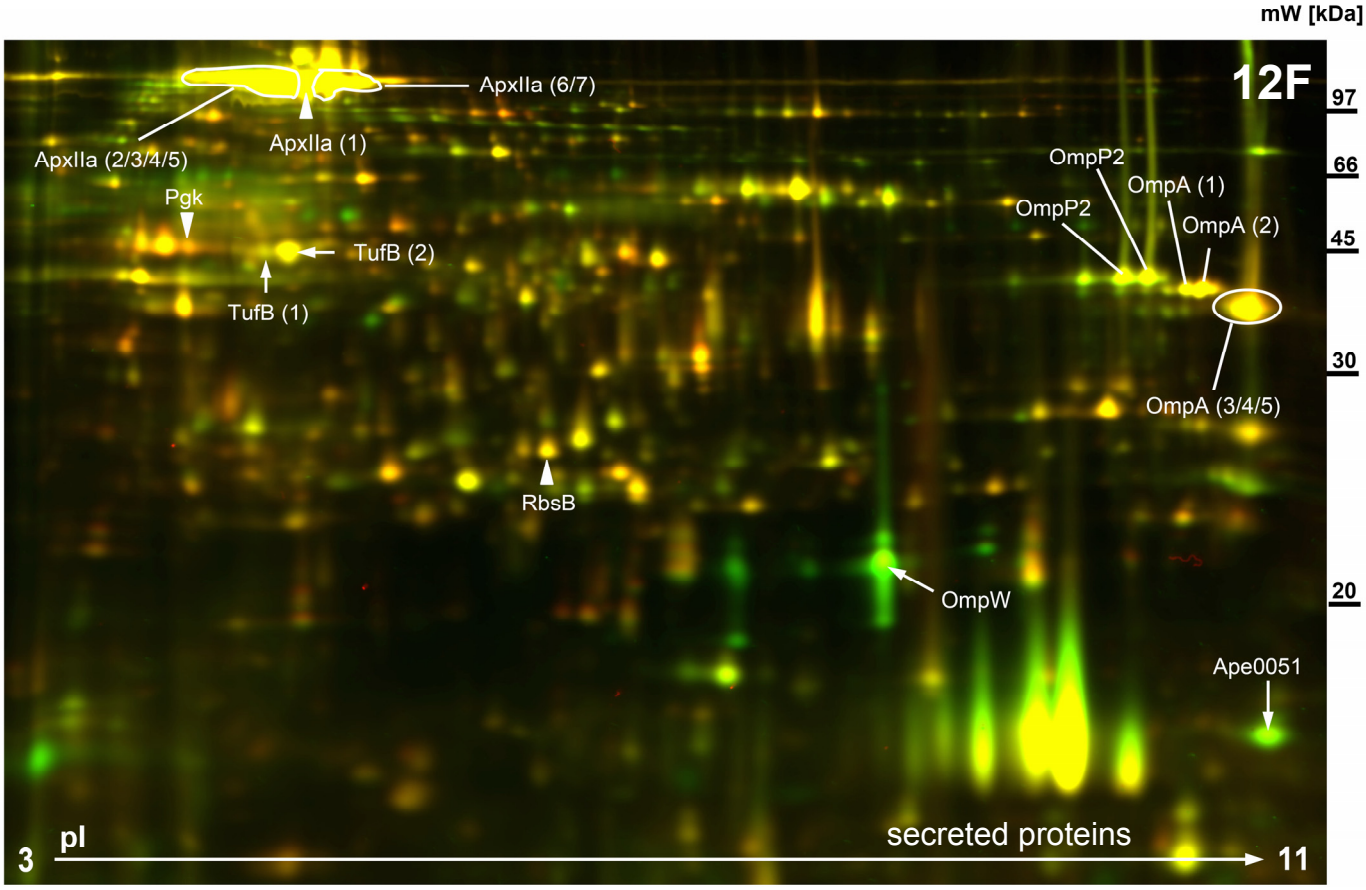

mW [kDa]
12F
ApxIIa (6/7)
ApxIIa (2/3/4/5)
ApxIIa (1)
Pgk
TufB (2)
TufB (1)
OmpP2
OmpA (1)
OmpP2
OmpA (2)
OmpA (3/4/5)
RbsB
OmpW
Ape0051
3
pI
secreted proteins
11
97
66
45
30
20

Fig. 12: **2D DIGE analysis of different protein preparations for comparison of protein expression between *A. pleuropneumoniae* wt (labelled with Cy5 [red]) and *A. pleuropneumoniae* Δ*arcA* (labelled with Cy3 [green]).** A, B and C, proteins precipitated directly from whole cell lysates. D, preparation of inner and outer membrane-associated proteins; E, preparation of outer membrane-associated proteins; F, preparation of secreted proteins. Isoelectric focussing was performed using Immobiline DryStrips with a pI gradient as shown on the respective gels. Second dimension separation of proteins was performed on 12.5 % polyacrylamide gels. For each protein preparation *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* were grown anaerobically in four independent cultures (A, B, C, D, F) or in three independent cultures (E) each. Using three fluorescent dyes, Cy2 (blue [internal standard]), Cy3 (green [either wt or Δ*arcA*]) and Cy5 (red [either wt or Δ*arcA*]) on each gel an internal standard and a protein preparation *of A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* were separated simultaneously. The fluorescence was detected using a Typhoon™ Trio fluorescence scanner. Quantitative and statistical analysis of differences in protein expression was performed with the DeCyder™ 2D 6.5 software. Each gel shown here represents one out of six gels (A, B, C, D, F) or one out of four gels (E) that were run and analyzed for the respective protein preparation. On all gels shown here *A. pleuropneumoniae* wt proteins are shown as red signals (Cy5) and *A. pleuropneumoniae* Δ*arcA* proteins are shown by green fluorescence (Cy3). Arrow heads highlight protein spots that were significantly ($p \leq 0.05$) upregulated by ArcA. Arrows indicate protein spots that were significantly ($p \leq 0.05$) downregulated by ArcA. Some protein spots are stressed by a line. These spots were not significantly regulated by ArcA. Protein spots of interest were excised from preparative gels, treated with trypsin for in gel digestion of proteins and, after recovery of peptides from the gel, analyzed by mass spectrometry.

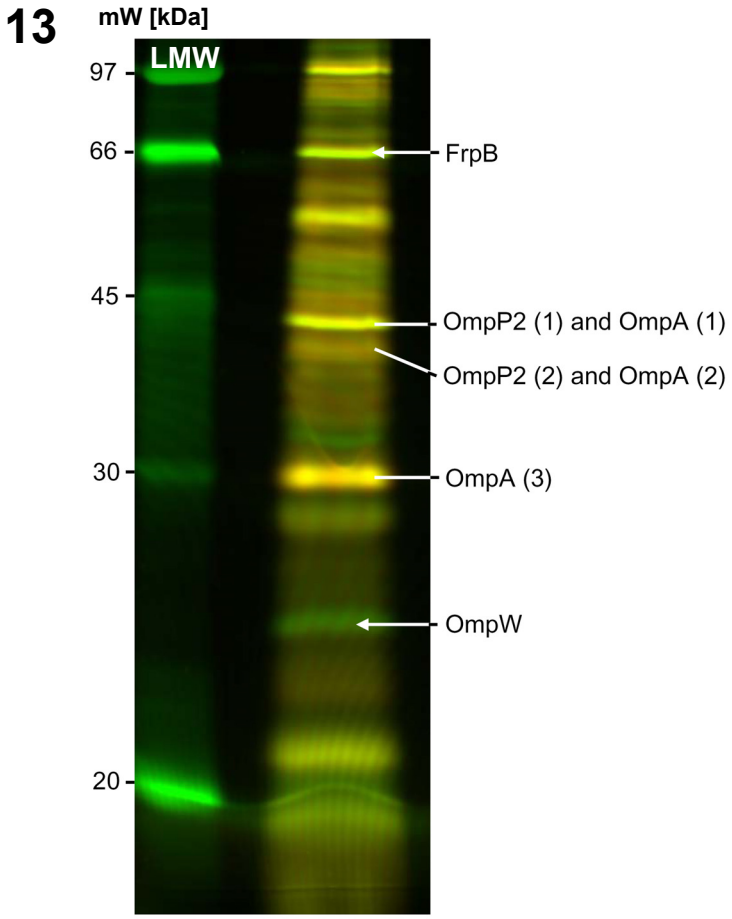


Fig. 13: **PAGE of an outer membrane protein preparation of *A. pleuropneumoniae* wt (labelled with Cy5 [red]) and *A. pleuropneumoniae* Δ*arcA* (labelled with Cy3 [green]).** Both preparations were mixed and separated in one lane. Fluorescence was detected using a Typhoon™ Trio fluorescence scanner. From *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* four independent cultures were prepared under anaerobic growth conditions and separate outer membrane protein preparations were performed. After labelling with different dyes, outer membrane proteins of *A. pleuropneumoniae* wt were mixed with outer membrane proteins of *A. pleuropneumoniae* Δ*arcA* resulting in four pairs. These were separated in four lanes on the same gel. Using the Fragment Analysis™ 2.1 Software band intensities were calculated for the protein bands according to their fluorescence intensity. Including the four biological repeats a statistical analysis was performed to detect significant differences in band intensities between the wt and the mutant strain. Protein bands that were identified as significantly ($p \leq 0.05$) downregulated by ArcA are highlighted by arrows. Bands of interest were excised and used for protein analysis by mass spectrometry.
LMW: Cy3 labelled low molecular weight marker (Amersham).

Table 3: Overview protein preparations, DIGE results and mass spectrometry results.

figure	protein preparation	IPG strip pI gradient	no. of differentially expressed spots [a]		no. of proteins identified by mass spectrometry		no. of different proteins identified	
			up in wt	up in Δ*arcA*	up in wt	up in Δ*arcA*	up in wt	up in Δ*arcA*
12A	whole cell lysate [b]	4 to 7	42	37	15 (4) [e]	21 (16) [e]	13	12
12B	whole cell lysate [c]	4 to 7	34	33	12 (4) [e]	20 (16) [e]	6	11
12C	whole cell lysate	7 to 11 NL[d]	3	7	0	5	0	4
12D	inner and outer membrane-associated	3 to 11 NL[d]	50	81	21	30	12	14
12E	outer membrane-associated	3 to 11 NL[d]	40	22	11	8	9	6
12F	secreted	3 to 11 NL[d]	8	13	3	4	3	3
13	outer membrane	–	–	–	0	2	0	2
	total	–	**177**	**193**	**62 (58) [f]**	**90 (74) [f]**	**30**	**29**
					152 (132) [f]		**58 [g]**	

a) obtained by 2D DIGE as statistical significant (Student's T-test $p \leq 0.05$)

b) protein precipitation with 10 % TCA

c) protein precipitation with 15 % TCA

d) non linear

e) the number in parenthesis indicates the number of spots that were found on both gels (12A and 12B). These proteins were identified once by mass spectrometry.

f) the number in parenthesis is the number of protein spots that were analyzed by mass spectrometry.

g) 30 different proteins upregulated plus 29 different proteins downregulated minus 1 protein (FrpB) up- and downregulated by ArcA dependent on protein preparation method

Table 4: Proteins significantly upregulated by ArcA.

spot [a]	protein [b]	T-test [c]	ratio [d]	MS # [e]	gel # [f]
whole cell lysates (Fig. 12A)					
GdhA	NADP-specific glutamate dehydrogenase	0.0019	**2.33**	M79	4
PtsI (1)	phosphoenolpyruvate protein phosphotransferase	7.50E-05	**1.63**	211	6
PflB (1)	formate acetyltransferase	0.0018	**1.45**	15	3
EngD	GTP dependent nucleic acid binding protein EngD	0.0077	**1.43**	225	6
Pgk	phosphoglycerate kinase	0.024	**1.38**	226	6
PtsI (2)	phosphoenolpyruvate protein phosphotransferase	0.00031	**1.37**	27	3
PtsI (3)	phosphoenolpyruvate protein phosphotransferase	0.00043	**1.29**	218	6
GlmS	glucosamine fructose 6 phosphate aminotransferase isomerizing	0.0094	**1.29**	49	3
CbiK	putative periplasmic binding protein CbiK	0.00072	**1.26**	10	2
DnaK	chaperone protein dnaK	0.0053	**1.21**	212	6
GpmA	2 3 bisphosphoglycerate dependent phosphoglycerate mutase	0.0063	**1.2**	9	2
SodA	manganese superoxide dismutase	0.04	**1.17**	5	2
Ape1259	hypothetical protein APL 1167	0.043	**1.16**	213	6
DeoC	deoxyribose phosphate aldolase	0.035	**1.15**	215	6
Adk	adenylate kinase	0.034	**1.14**	222	6
whole cell lysates (Fig. 12B)					
Ape0761 (1)	putative methylation subunit type III restriction modification system	0.00061	**7.13**	210	6
Ape0761 (2)	putative methylation subunit type III restriction modification system	0.00096	**5.27**	209	6
GlmS	glucosamine fructose 6 phosphate aminotransferase isomerizing	0.047	**1.53**	49	3
PflB (1)	formate acetyltransferase	0.0021	**1.51**	15	3
PflB (2)	formate acetyltransferase	0.018	**1.47**	17	3
PflB (3)	formate acetyltransferase	0.0018	**1.46**	22	3
PflB (4)	formate acetyltransferase	0.029	**1.42**	21	3
PflB (5)	formate acetyltransferase	0.0022	**1.4**	18	3
PtsI (1)	phosphoenolpyruvate protein phosphotransferase	0.04	**1.37**	27	3
PtsI (2)	phosphoenolpyruvate protein phosphotransferase	0.039	**1.34**	218	6
TorZ	trimethylamine N oxide reductase precursor	0.0034	**1.3**	61	7
AckA	acetate kinase	0.029	**1.09**	36	3
inner and outer membrane-associated proteins (Fig. 12D)					
FrpB	iron regulated outer membrane protein B	0.011	**6.66**	260	11
OmpA (1)	outer membrane protein P5 precursor	0.004	**6.65**	258	11
OmpA (2)	outer membrane protein P5 precursor	0.0035	**6.55**	255	11
OmpA (3)	outer membrane protein P5 precursor	0.0038	**6.54**	256	11
Ape0761	putative methylation subunit type III restriction modification system	0.00011	**5.96**	267	11
OmpP2 (1)	outer membrane protein P2 precursor OMP P2	0.016	**5.05**	254	11
OmpP2 (2)	outer membrane protein P2 precursor OMP P2	0.014	**4.42**	253	11
NapA (1)	periplasmic nitrate reductase precursor	0.047	**3.24**	263	11
Adh2	aldehyde-alcohol dehydrogenase 2	0.033	**2.34**	M55	11
ApxIIA	RTX II toxin determinant A	0.027	**2.32**	275	11
TorZ (1)	trimethylamine-N-oxide reductase precursor	0.035	**1.9**	M56	11
DmsA (1)	anaerobic dimethyl sulfoxide reductase chain A precursor	0.017	**1.85**	273	11
DmsA (2)	anaerobic dimethyl sulfoxide reductase chain A precursor	0.0075	**1.77**	274	11
PflB (1)	formate acetyltransferase	0.00074	**1.72**	M42	11
PflB (2)	formate acetyltransferase	5.90E-05	**1.7**	M41	11
PflB (3)	formate acetyltransferase	0.0031	**1.68**	287	11
PykA	pyruvate kinase	0.016	**1.68**	262	11

PflB (4)	formate acetyltransferase	2.10E-06	**1.67**	M40	11
GlnA	Glutamine synthetase	0.021	**1.62**	244	11
TorZ (2)	trimethylamine-N-oxide reductase precursor	0.032	**1.57**	M57	11
PflB (5)	formate acetyltransferase	1.50E-05	**1.53**	268	11
outer membrane-associated proteins (Fig. 12E)					
OmpA (1)	outer membrane protein P5 precursor	0.045	**5.92**	282	13
OmpA (2)	outer membrane protein P5 precursor	0.0054	**5.63**	M29	13
ApxIIa (1)	RTX-II toxin determinant A	0.052	**5.37**	M13	13
OmpP2 (1)	outer membrane protein P2 precursor OMP P2	0.044	**2.92**	278	12
OmpP2 (2)	outer membrane protein P2 precursor OMP P2	0.053	**2.71**	279	12
PtsH	PTS system phosphocarrier protein HPr	0.034	**1.91**	M28	13
ZnuA	high-affinity zinc uptake system protein ZnuA precursor	0.047	**1.47**	M26	13
GapA	glyceraldehyde-3-phosphate dehydrogenase	0.038	**1.46**	M27	13
AckA	acetate kinase	0.0033	**1.45**	235	13
Efp	Elongation factor P	0.033	**1.4**	M25	13
TorZ	trimethylamine-N-oxide reductase precursor	0.003	**1.3**	M21	13
secreted proteins (Fig. 12F)					
RbsB	D ribose binding periplasmic protein precursor	0.037	**1.45**	133	14
ApxIIa (1)	RTX-II toxin determinant A	0.044	**1.23**	M05	14
Pgk	phosphoglycerate kinase	0.035	**1.14**	M11	14

Table 5: Proteins significantly downregulated by ArcA.

spot [a]	protein [b]	T-test [c]	ratio [d]	MS # [e]	gel # [f]
whole cell lysates (Fig. 12A)					
Fbp (1)	fructose 1 6 bisphosphatase	2.00E-08	**-8.49**	2	1
AldA (1)	putative aldehyde dehydrogenase aldA	3.10E-06	**-6.9**	60	5
YkgE	putative dehydrogenase subunit	1.60E-07	**-5.9**	4	1
AldA (2)	putative aldehyde dehydrogenase aldA	0.011	**-4.05**	40	3
AspA (1)	aspartate ammonia lyase	0.00029	**-3.69**	62	5
AceE (1)	pyruvate dehydrogenase E1 component	1.50E-05	**-3.65**	30	3
AdhI	alcohol dehydrogenase 1	4.80E-07	**-3.62**	12	2
AceE (2)	pyruvate dehydrogenase E1 component	5.60E-07	**-3.61**	16	3
AceE (3)	pyruvate dehydrogenase E1 component	7.00E-05	**-3.13**	M75	4
LpdA (1)	dihydrolipoyl dehydrogenase	2.50E-08	**-2.85**	13	2
LpdA (2)	dihydrolipoyl dehydrogenase	2.40E-07	**-2.82**	42	3
AceE (4)	pyruvate dehydrogenase E1 component	3.30E-06	**-2.59**	19	3
Ape0477	hypothetical protein APL 0444	0.0053	**-2.42**	3	1
AceE (5)	pyruvate dehydrogenase E1 component	5.30E-05	**-2.27**	M77	4
AspA (2)	aspartate ammonia lyase	0.018	**-2.06**	55	3
AspA (3)	aspartate ammonia lyase	7.80E-05	**-1.62**	228	6
PckA (1)	phosphoenolpyruvate carboxykinase ATP	0.00036	**-1.43**	33	3
Pnp (1)	polyribonucleotide nucleotidyltransferase	0.023	**-1.43**	220	6
Mtn	MTA SAH nucleosidase	0.0021	**-1.39**	221	6
Pnp (2)	polyribonucleotide nucleotidyltransferase	0.016	**-1.34**	24	3
TktA	transketolase 2	0.039	**-1.33**	38	3
whole cell lysates (Fig. 12B)					
AldA (2)	putative aldehyde dehydrogenase aldA	0.00019	**-10.99**	40	3
Fbp (1)	fructose 1 6 bisphosphatase	0.00038	**-8.05**	2	1
Fbp (2)	fructose 1 6 bisphosphatase	0.00019	**-4.12**	59	5
AldA (1)	putative aldehyde dehydrogenase aldA	0.0017	**-4.05**	60	5

AceE (1)	pyruvate dehydrogenase E1 component	0.00029	**-3.12**	30	3
AceF	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	0.00065	**-2.99**	23	3
LpdA (1)	dihydrolipoyl dehydrogenase	0.00018	**-2.97**	13	2
AceE (2)	pyruvate dehydrogenase E1 component	0.00067	**-2.88**	16	3
LpdA (2)	dihydrolipoyl dehydrogenase	0.0022	**-2.78**	42	3
YkgE	putative dehydrogenase subunit	0.0028	**-2.65**	4	1
AdhI	alcohol dehydrogenase 1	0.00018	**-2.54**	12	2
MaeB	NADP dependent malic enzyme NADP ME	0.011	**-2.39**	1	1
AceE (3)	pyruvate dehydrogenase E1 component	0.0017	**-2.33**	M75	4
AspA (1)	aspartate ammonia lyase	0.0035	**-1.66**	62	5
AspA (2)	aspartate ammonia lyase	0.047	**-1.63**	55	3
AceE (4)	pyruvate dehydrogenase E1 component	0.0037	**-1.58**	19	3
PckA (1)	phosphoenolpyruvate carboxykinase ATP	0.0022	**-1.42**	33	3
PckA (2)	phosphoenolpyruvate carboxykinase ATP	0.018	**-1.42**	44	3
Pnp (1)	polyribonucleotide nucleotidyltransferase	0.029	**-1.27**	24	3
Pnp (2)	polyribonucleotide nucleotidyltransferase	0.011	**-1.25**	220	6
whole cell lysates (Fig. 12C)					
YkgF (1)	putative electron transport protein	0.0011	**-11.19**	172	9
YkgF (2)	putative electron transport protein	1.10E-05	**-10.08**	174	9
Mqo	putative malate quinone oxidoreductase	0.0074	**-7.49**	170	9
RplF	50S ribosomal protein L6	0.032	**-2.01**	202	10
Ape0051	hypothetical protein APL 0049	0.05	**-1.81**	205	10
inner and outer membrane-associated proteins (Fig. 12D)					
Fbp	fructose 1 6 bisphosphatase	5.30E-06	**-10.12**	266	11
AceF (1)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	4.00E-06	**-4.51**	237	11
AceF (2)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	6.70E-06	**-4.3**	M32	11
AceF (3)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	3.00E-05	**-4.11**	M33	11
AceF (4)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	3.20E-05	**-3.88**	M30	11
AceF (5)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	4.80E-05	**-3.68**	236	11
AceF (6)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	1.20E-05	**-3.66**	M31	11
AceE (1)	pyruvate dehydrogenase E1 component	5.10E-06	**-3.53**	M39	11
TktA	transketolase 2	1.90E-05	**-3.24**	M36	11
LpdA (1)	dihydrolipoyl dehydrogenase	8.60E-06	**-3.12**	M45	11
AceE (2)	pyruvate dehydrogenase E1 component	3.60E-05	**-2.98**	M38	11
AceF (7)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	0.00017	**-2.98**	283	11
LpdA (2)	dihydrolipoyl dehydrogenase	1.80E-05	**-2.88**	M44	11
LpdA (3)	dihydrolipoyl dehydrogenase	0.00066	**-2.6**	288	11
LpdA (4)	dihydrolipoyl dehydrogenase	0.00026	**-2.56**	290	11
LpdA (5)	dihydrolipoyl dehydrogenase	0.00011	**-2.38**	291	11
RpsA (1)	30S ribosomal protein S1	0.038	**-2.3**	239	11
RpsA (2)	30S ribosomal protein S1	0.049	**-2.1**	M34	11
AceE (3)	pyruvate dehydrogenase E1 component	0.00041	**-2.07**	M37	11
Pnp	polyribonucleotide nucleotidyltransferase	0.011	**-1.97**	238	11
AsnA	aspartate ammonia ligase	0.011	**-1.79**	299	11
PckA (1)	phosphoenolpyruvate carboxykinase ATP	0.01	**-1.69**	M35	11
AtpD	ATP synthase subunit beta	0.032	**-1.68**	240	11
MrsA	phosphoglucosamine mutase	0.0012	**-1.67**	M43	11
GalE (1)	UDP glucose 4 epimerase	0.012	**-1.65**	296	11
PckA (2)	phosphoenolpyruvate carboxykinase ATP	0.011	**-1.6**	252	11

Ape1591 (1)	hybrid peroxiredoxin HyPrx5	0.036	**-1.59**	242	11
TufB	Elongation factor Tu	0.0041	**-1.57**	248	11
Ape1591 (2)	hybrid peroxiredoxin HyPrx5	0.034	**-1.57**	243	11
GalE (2)	UDP glucose 4 epimerase	0.014	**-1.5**	295	11
outer membrane-associated proteins (Fig. 12E)					
Fbp (1)	fructose 1 6 bisphosphatase	0.0034	**-4.53**	M23	13
MaeB (1)	NADP dependent malic enzyme NADP ME	0.00087	**-4.38**	231	13
Fbp (2)	fructose 1 6 bisphosphatase	0.002	**-4.08**	M24	13
MaeB (2)	NADP dependent malic enzyme NADP ME	0.0094	**-2.14**	M22	13
YkgE	putative dehydrogenase subunit	0.019	**-1.68**	234	13
PtrA	protease 3 precursor	0.0035	**-1.34**	M20	13
PckA	phosphoenolpyruvate carboxykinase ATP	0.035	**-1.23**	M18	13
Prc	tail-specific protease precursor	0.043	**-1.13**	M19	13
secreted proteins (Fig. 12F)					
OmpW	outer membrane protein W precursor	0.000041	**-6.27**	122	14
Ape0051	hypothetical protein APL 0049	0.023	**-2.16**	130	14
TufB (1)	Elongation factor Tu	0.033	**-1.86**	148	14
TufB (2)	Elongation factor Tu	0.035	**-1.86**	M09	14
outer membrane proteins (Fig. 13)					
OmpW	outer membrane protein W precursor	0.01	**-6.83**	78	16
FrpB	iron regulated outer membrane protein	0.046	**-1.88**	75	16

Table 6: Selected proteins not significantly up- or downregulated by ArcA.

spot [a]	protein [b]	T-test [c]	ratio [d]	MS # [e]	gel # [f]
whole cell lysates (Fig. 12C)					
NapA (1)	periplasmic nitrate reductase precursor	0.54	**-1.06**	175	9
NapA (2)	periplasmic nitrate reductase precursor	0.98	**-1**	168	10
OmpP2 (1)	outer membrane protein P2 precursor OMP P2	0.23	**1.22**	183	10
OmpP2 (2)	outer membrane protein P2 precursor OMP P2	0.28	**1.31**	184	10
OmpA (1)	outer membrane protein P5 precursor	0.38	**1.19**	185	10
OpmA (2)	outer membrane protein P5 precursor	0.38	**1.21**	187	10
FrpB	iron regulated outer membrane protein B	0.24	**-1.52**	173	10
inner and outer membrane-associated membrane proteins (Fig. 12D)					
NapA (2)	periplasmic nitrate reductase precursor	0.057	**2.81**	264	11
OmpA (4)	outer membrane protein P5 precursor OMP P5	0.093	**2.74**	261	11
PpiI	50S ribosomal protein L9	0.076	**-10.44**	269	11
outer membrane-associated proteins (Fig. 12E)					
ApxIIa (2)	RTX II toxin determinant A	0.22	**4.26**	230	13
ApxIIa (3)	RTX II toxin determinant A	0.21	**4.67**	281	13
secreted proteins (Fig. 12F)					
OmpP2 (1)	outer membrane protein P2 precursor OMP P2	0.082	**-1.24**	149	14
OmpP2 (2)	outer membrane protein P2 precursor OMP P2	0.059	**-1.26**	150	14
OmpA (1)	outer membrane protein P5 precursor	0.079	**1.19**	161	14
OmpA (2)	outer membrane protein P5 precursor	0.23	**1.17**	151	14
OmpA (3)	outer membrane protein P5 precursor OMP P5	0.14	**1.25**	165	14
OmpA (4)	outer membrane protein P5 precursor OMP P5	0.14	**1.25**	163	14
OmpA (5)	outer membrane protein P5 precursor OMP P5	0.14	**1.25**	153	14
ApxIIa (2)	RTX-II toxin determinant A	nd	**nd**	M01	14
ApxIIa (3)	RTX-II toxin determinant A	nd	**nd**	M02	14
ApxIIa (4)	RTX-II toxin determinant A	nd	**nd**	M03	14

ApxIIa (5)	RTX-II toxin determinant A	0.13	**1.21**	M04	14
ApxIIa (6)	RTX-II toxin determinant A	0.072	**1.28**	M06	14
ApxIIa (7)	RTX-II toxin determinant A	0.078	**1.28**	M07	14
outer membrane proteins (Fig. 13)					
OmpA (2)	outer membrane protein P5 precursor OMP P5; outer membrane protein P5 precursor	0.48	**1.51**	92, 93	17
OmpP2 (2)	outer membrane protein P2 precursor OMP P2	0.48	**1.51**	94	17
OmpP2 (1)	outer membrane protein P2 precursor OMP P2	0.34	**1.49**	73	15
OmpA (1)	outer membrane protein P5 precursor; outer membrane protein P5 precursor OMP P5	0.34	**1.49**	84, 82	16
OmpA (3)	outer membrane protein P5 precursor; outer membrane protein P5 precursor OMP P5	0.39	**1.21**	79, 80	16

a) name of protein spot on gel (Fig. 12 A-F, 13)

b) result of mass spectrometry analysis.

c) statistical analysis using unpaired Student's T-test

d) ratio between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* ∆*arcA*. Positive values indicate upregulation by ArcA, negative values indicate downregulation by ArcA.

e) number of the mass spectrometric analysis (Appendix G 6) that was performed for the identification of the protein of interest from the respective preparative gel [f]. Simple numbers indicate analysis by Q-TOF MSMS; numbers beginning with the capital letter M indicate analysis by MALDI-TOF MS.

f) this number indicates the preparative gel (Appendix G 5) from which the respective protein spot was obtained.

D 2.2.1 Analysis of differentially expressed proteins

Several enzymes catalyzing reactions of glycolysis or gluconeogenesis and at the interface of these pathways to the citric acid cycle were found to be affected anaerobically by ArcA using 2D DIGE and subsequent mass spectrometry. A protein homologue to the UDP glucose-4-epimerase (GalE) necessary for introduction of galactose into the glycolysis was identified as upregulated by ArcA in *A. pleuropneumoniae*. The glycolysis enzymes glyceraldehyde-3-phosphate dehydrogenase (GapA), 3-phosphoglycerate kinase (Pgk) and the 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase (GmpA) were identified by 2D DIGE of either whole cell lysate proteins, outer membrane-associated proteins or secreted proteins (Fig. 12A, 12E, 12F) to be slightly upregulated by ArcA of *A. pleuropneumoniae*. The pyruvate dehydrogenase complex catalyzes the irreversible transformation of the last glycolysis metabolite pyruvate into acetyl-CoA in order to introduce it into the citric acid cycle. The components of the pyruvate dehydrogenase protein complex, pyruvate dehydrogenase E1 component (AceE), dihydrolipoyllysine residue acetyltransferase (AceF) and dihydrolipoyl dehydrogenase (LpdA) were identified on gels of whole cell lysates (Fig. 12A, 12B) and on gels of inner and outer membrane-associated proteins (Fig. 12D). They were strongly downregulated by ArcA. Of each subunit of the pyruvate dehydrogenase

complex several protein spots were identified on these gels presumably due to differences in residual secondary structure or posttranslational modifications. The phosphoenolpyruvate carboxykinase (PckA) initiating gluconeogenesis from the citric acid cycle intermediate oxaloacetate was also identified to be downregulated by ArcA. The protein PckA was identified as differentially expressed in several DIGE experiments (Fig. 12A, 12B, 12D, 12E) and sequenced from the respective preparative gels. The NADP dependent malic enzyme (MaeB) is another enzyme at the interface of glycolysis and citric acid cycle that has been identified to be downregulated by ArcA in *A. pleuropneumoniae*. DIGE analysis of whole cell lysates (Fig. 12B) and outer membrane-associated proteins (Fig. 12E) led to the identification of MaeB as severely downregulated by ArcA. The protein was identified by mass spectrometry from the respective preparative gels. A key enzyme of gluconeogenesis, the fructose-1,6-bisphosphatase (Fbp), was also identified as downregulated by ArcA. This protein was identified by DIGE analysis and sequenced on gels of whole cell lysates (Fig. 12A, 12B) inner membrane preparations (Fig. 12D) and on outer membrane-associated protein preparations (Fig. 12E). The pyruvate kinase (PykA) transforming phosphoenolpyruvate into pyruvate and the formate acetyltransferase (PflB) catalyzing the transformation of pyruvate and CoA into formate and acetyl-CoA were identified as upregulated by ArcA in *A. pleuropneumoniae*. PykA was found by 2D DIGE analyses of inner and outer membrane-associated proteins (Fig. 12D), and PflB was identified on DIGE gels of whole cell lysates (Fig. 12A, 12B) and on gels of inner and outer membrane-associated proteins (Fig. 12D). The acetate kinase (AckA) generating ATP by dephosphorylation of acetylphosphate is upregulated by ArcA as identified by 2D DIGE of whole cell lysates and outer membrane associated proteins (Fig. 12B, 12E).

The alcohol dehydrogenase 1 (AdhI) which is a homologue to the *E. coli* protein AdhP was identified to be downregulated by ArcA. AdhI was identified by 2D DIGE of gels on which protein preparations obtained from whole cell lysates were separated (Fig. 12A, 12B). The putative aldehyde dehydrogenase AldA was also identified on gels of whole cell lysates (Fig. 12A, 12B) as strongly downregulated by ArcA in *A. pleuropneumoniae*. In contrast, the aldehyde-alcohol dehydrogenase 2 *of A. pleuropneumoniae* wt was found to be upregulated by ArcA on 2D DIGE gels of inner and outer membrane associated proteins (Fig. 12D).

The putative dehydrogenase subunit YkgE was found to be downregulated by ArcA on gels of whole cell lysates as well as on gels where outer membrane-associated proteins were separated (Fig. 12A, 12B, 12E). The putative electron transport protein YkgF forming a putative operon with YkgE was also identified as strongly downregulated by ArcA in DIGE analyses of whole cell lysates (Fig. 12C). On the same gel a putative malate quinine reductase (Mqo) was identified as strongly downregulated by ArcA. 2D DIGE revealed that the anaerobic dimethyl reductase chain A (DmsA) and the trimethylamine N-oxide reductase

(TorZ) were upregulated by ArcA. DmsA and TorZ were identified by 2D DIGE of inner and outer membrane-associated proteins (Fig. 12D, 12E) with TorZ additionally found by 2D DIGE of whole cell lysates (Fig. 12B). The elongation factor Tu (TufB) is upregulated by ArcA in *A. pleuropneumoniae* and was identified by 2D DIGE of gels from inner and outer membrane-associated proteins as well as on preparation of secreted proteins (Fig. 12D, 12F). The translation elongation factor P (Efp) was found to be upregulated by ArcA by 2D DIGE of outer membrane-associated proteins (Fig. 12E)

2D DIGE of whole cell lysates (Fig. 12A, 12B) revealed that the phosphoenolpyruvate protein phosphotransferase (PtsI) of *A. pleuropneumoniae* was upregulated by ArcA. The HPr-related protein (PtsH) which is the second of two sugar-non-specific protein constituents of the phosphotransferase system was identified as upregulated by ArcA in 2D DIGE of outer membrane-associated proteins (Fig. 12E).

The putative methylation subunit type III restriction modification system (Ape0761) was identified on gels of whole cell lysates and on gels of inner and outer membrane-associated proteins (Fig. 12B, 12D) as strongly upregulated by ArcA.

The 30S ribosomal protein S1 was found by 2D DIGE of inner and outer membrane-associated proteins as downregulated by ArcA in *A. pleuropneumoniae* (Fig. 12D). On the same gel the 50S ribosomal protein (RplI) was found to be more then 10 fold downregulated by ArcA; however, this spot slightly failed the significance threshold of the DIGE analysis. A further ribosomal protein, the 50S ribosomal protein L6 (RplF), was identified by DIGE on gels of whole cell lysates (Fig. 12C) as downregulated by ArcA in *A. pleuropneumoniae*.

On gels of secreted proteins (Fig. 12F) the outer membrane protein W (OmpW) appeared as strongly downregulated by ArcA in *A. pleuropneumoniae*. On the same gel the hypothetical protein Ape0051 was also identified as downregulated by ArcA. The gene encoding this protein exhibiting homology to an outer membrane protein of *A. actinomycetemcomitans* is located just downstream of *arcA*.

D 2.2.2 Comparison between detergent and non detergent-dependent protein preparations

Several differentially expressed proteins where identified by 2D DIGE of different protein preparations and analyzed by mass spectrometry from the respective preparative gels. In most cases calculated differences in expression between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* were similar (Table 4, 5, 6). However, for some proteins the 2D DIGE-based comparison of protein expression between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* differed considerably depending on the method of protein

preparation; thus, differences were observed between the detergent-based protein preparations of inner and outer membrane-associated proteins (Fig. 12D) and outer membrane associated proteins (Fig. 12E) on the one hand and the non detergent-based preparations of whole cell lysates (Fig. 12A, 12B, 12C) and secreted proteins (Fig. 12F) on the other hand.

The outer membrane protein P2 (OmpP2) and the outer membrane protein P5 (OmpA) were found by 2D DIGE of inner and outer membrane-associated proteins (Fig. 12D) as more then 5-fold upregulated by ArcA. 2D DIGE analysis of outer membrane-associated proteins (Fig. 12E) also revealed a significant upregulation of the outer membrane proteins OmpP2 and OmpA. However, OmpP2 and OmpA where also identified on 2D DIGE gels of whole cell lysates (Fig. 12C). In these preparations, both proteins appeared only slightly and not statistically significant upregulated by ArcA. OmpA was also identified by 2D DIGE of secreted proteins (Fig. 12F) as slightly but not significantly upregulated whereas OmpP2 even appeared slightly but not significantly downregulated by ArcA. A similar observation was made for the RTX-II toxin determinant A (ApxIIA) which was found to be strongly upregulated by ArcA in 2D DIGE analysis of inner and outer membrane-associated proteins (Fig. 12D) and outer membrane-associated proteins (Fig. 12E) whereas 2D DIGE analysis of secreted proteins (Fig. 12F) revealed only a slight (not significant) upregulation of ApxIIA. Additionally the iron-regulated outer membrane protein B (FrpB) and the periplasmic nitrate reductase (NapA) were identified by 2D DIGE of inner and outer membrane-associated proteins (Fig. 12D) as increased in *A. pleuropneumoniae* wt by 6.7-fold and 3.2-fold, respectively. Both proteins were also identified on gels of whole cell lysates (Fig. 12C) revealing a 1.5-fold (not significant) downregulation of FrpB by ArcA and no difference between both strains in expression of NapA.

D 2.2.3 Comparison of microarray and 2D DIGE analyses

Of 78 protein spots resembling 23 different proteins that were identified by 2D DIGE as statistically significantly downregulated by ArcA 22 genes were also identified as downregulated significantly by microarray analysis. Only the ATP synthase subunit beta was found by 2D DIGE analysis as 1.68-fold downregulated by ArcA and by microarray analysis as 1.23-fold upregulated. 2D DIGE revealed 40 protein spots as significantly upregulated by ArcA belonging to 17 different proteins for which significant results were also obtained by microarray analysis. However, whereas the microarray analysis showed the same kind of regulation as the 2D DIGE analysis for 12 genes, expression of 5 genes was found to be regulated in the opposite direction (downregulated by ArcA). These 5 genes relate to 8

protein spots that were identified as upregulated by ArcA in 2D DIGE; these five proteins include ApxIIA, FrpB, and NapA which were shown to be regulated differently depending on the mode of preparation (see above and Fig. 14).

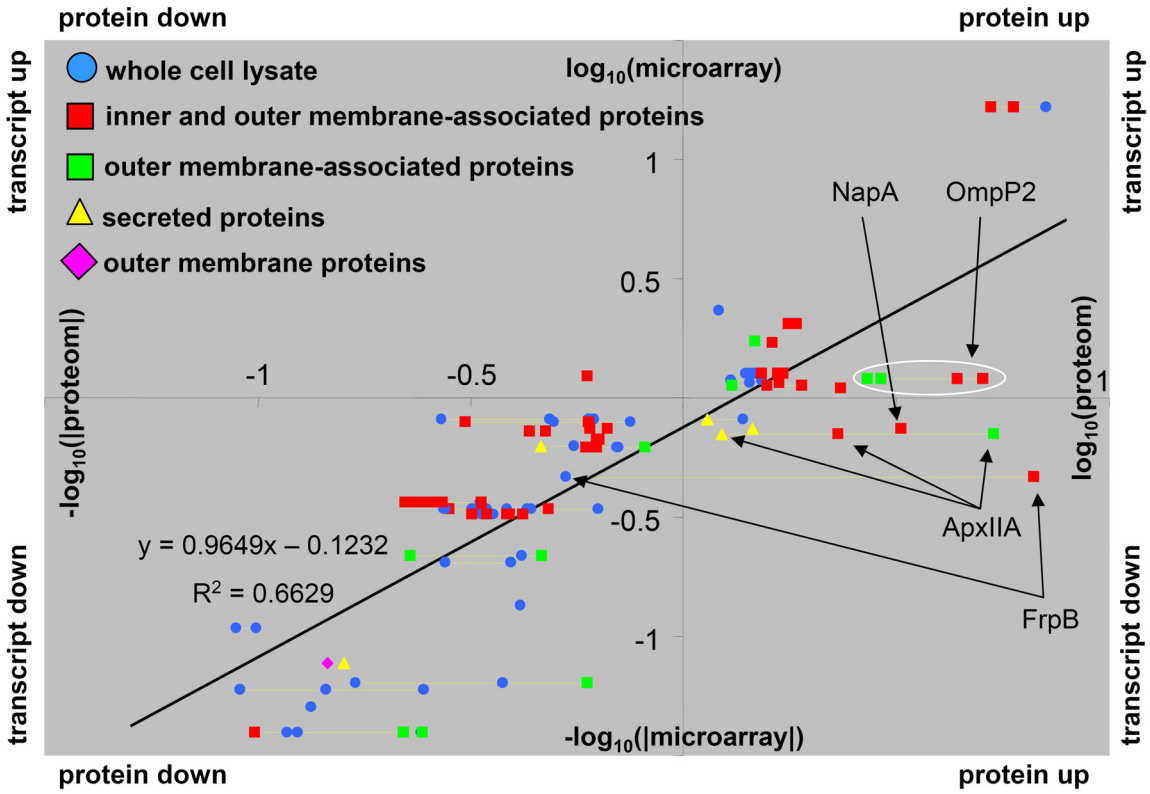


Fig. 14: Comparison of transcript (microarray) and protein (2D DIGE, quantitative PAGE) ratios between anaerobically grown *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA*. Transcripts as well as proteins that were identified to be upregulated due to ArcA got a positive value representing the factor of upregulation (Appendix G 1, Table 4). The $\log_{10}$ of these factors is represented by positive values on the y-axis for transcripts and positive values on the x-axis for proteins. Transcripts and proteins that were downregulated due to ArcA are indicated by a negative number representing the factor of downregulation (Appendix G 2, Table 5). The negative $\log_{10}$ of the absolute value is shown as negative value on the y-axis for transcripts and as negative values of the x-axis for proteins. Proteomics often led to multiple identification of the same protein. Symbols of a certain protein are connected by a yellow line. Dependent of the method of preparation different symbols were used as indicated in the diagram. The line of best fit was calculated showing a slope close to one. Proteins that deviated strongly from the line of best fit are indicated by arrows.

D 3 The HlyX regulon of *A. pleuropneumoniae*

D 3.1 Global transcription analysis of *A. pleuropneumoniae* Δ*hlyX*

A global gene expression profiling using a DNA microarray analysis was performed for the identification of HlyX-regulated genes. Six independent expression profiles of anaerobically grown *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX*, respectively, were analyzed and compared. The expression of 234 genes was upregulated more then 2-fold by HlyX, and among these the expression of 34 was increased more then 6-fold. An additional 164 genes were found to be upregulated by HlyX by a factor between 1.5 and 2. On the other hand 184 genes were found to by downregulated more then 2-fold by HlyX with none of these genes being downregulated more than 6-fold. In addition, 321 genes were downregulated by a factor between 1.5 and 2 by HlyX (Fig. 15, Appendix G 3 and G4).

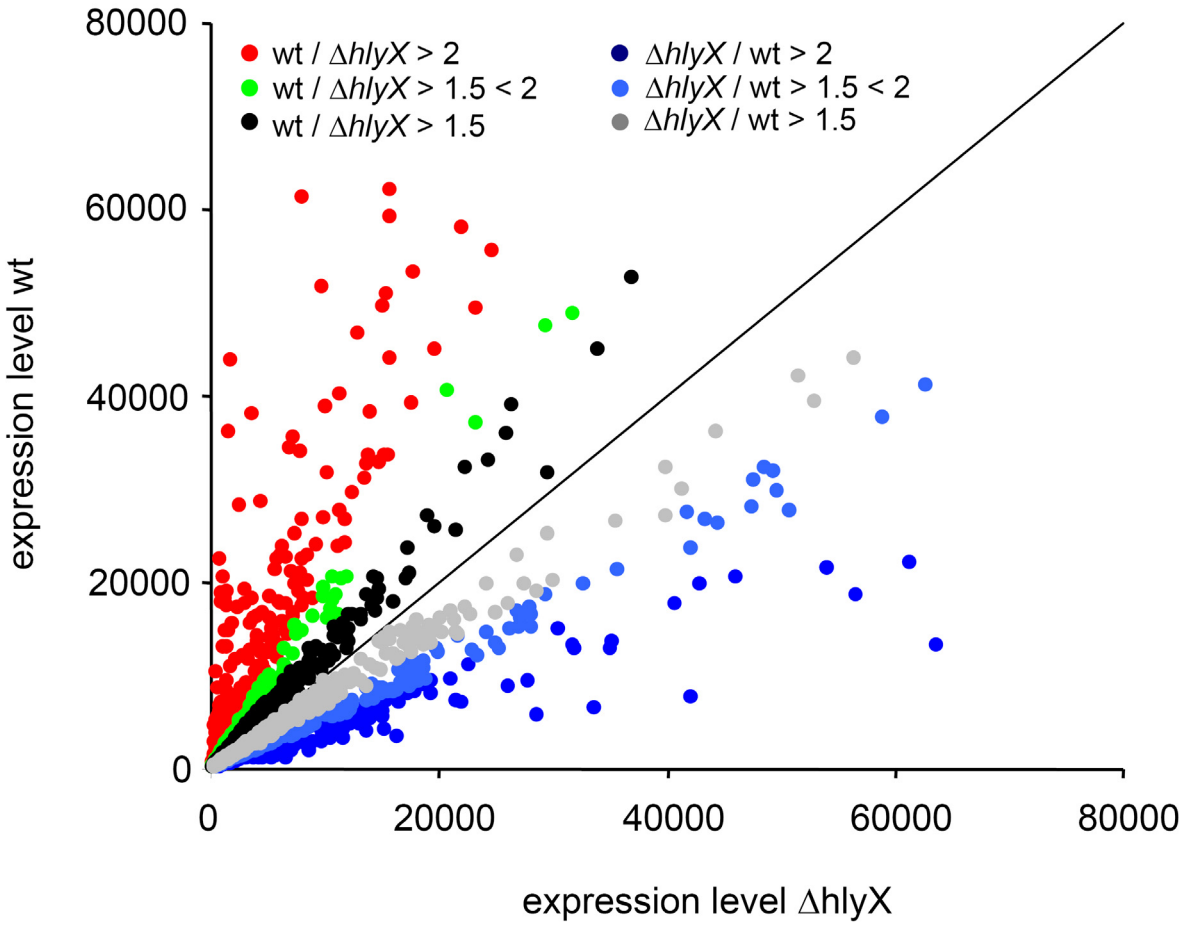


Fig. 15: Summary of microarray results wt vs. Δ*hlyX*. Each spot represents a gene that has been identified to be significantly affected by ArcA ($p \leq 0.05$). Spots appearing above the diagonal line represent genes that were upregulated by HlyX. Those located below the diagonal line exhibited a higher expression level in the *hlyX* deletion mutant caused by a downregulation due to HlyX in *A. pleuropneumoniae* wt. Green spots indicate genes upregulated by more then 1.5-fold and red spots indicate genes upregulated by more then 2-fold due to HlyX. Light blue spots indicate genes downregulated by more then 1.5-fold and dark blue spots indicate genes downregulated by more then 2-fold due to HlyX.

D 3.1.2 Functional classification of HlyX regulated genes

The 398 genes upregulated by HlyX by more then 1.5 fold and the 505 genes downregulated by HlyX by more than 1.5 fold were analyzed with respect to their functional classification using the database Cluster of Orthologous Groups of proteins (COGs) (http://www.ncbi.nlm.nih.gov/COG/; Tatussov et al., 1997). HlyX controlled the expression of genes belonging to almost all prokaryotic categories. In some categories differences between up- and downregulated genes were apparent; 51 genes belonging to the category “carbohydrate transport and metabolism (G)” were downregulated by HlyX compared to 11 genes of that category whose expression was upregulated. 25 genes of the category “nucleotide transport and metabolism (F)” were downregulated by HlyX compared to 6 genes that were upregulated. For the other categories the observed discrepancies between HlyX up- and downregulated genes were found to be less pronounced (Fig. 16).

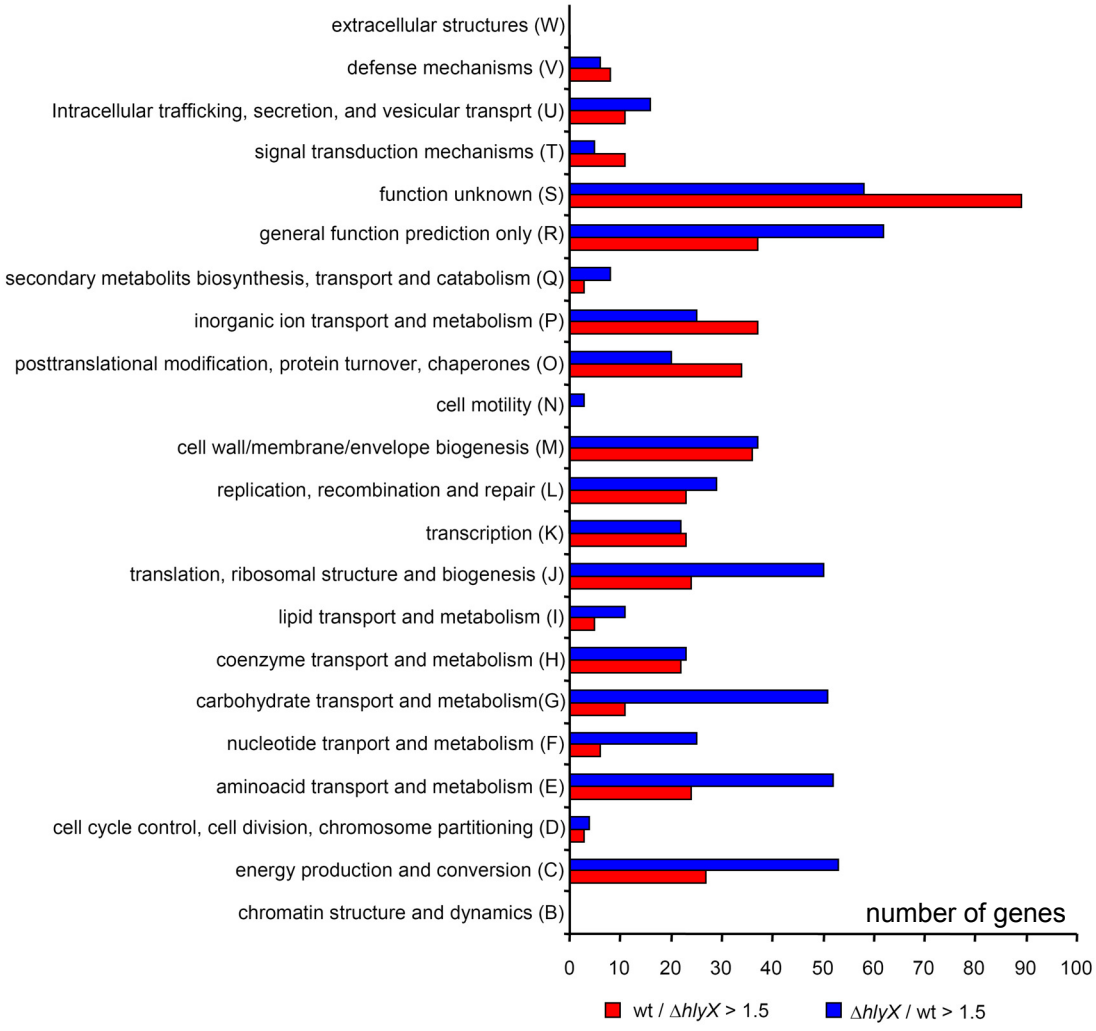


Fig. 16: Functional classification of HlyX regulated genes according to the database Cluster of Orthologous Groups of Proteins (COGs). The classification has been adopted from the "Simple Yet Powerful Genome Browser" database for *A. pleuropneumoniae* Ser 5 L20 (http://informatics.bio.nrc.ca/ap5b)

D 3.1.2 Analysis of the HlyX regulon

The gene *ape0761* was identified as strongest upregulated by HlyX by a factor of almost 30. It encodes for a putative methylation subunit of a type III restriction-modification system. The adjacent gene downstream of *ape0761*, *ape0760* shared homology to a potential type I restriction enzyme, a DEAD/DEAH box helicase domain protein. Expression of *ape0760* was increased nearly 10-fold. Expression of the next gene downstream, *ape0758*, encoding for a hypothetical ATP-dependent helicase was increased 8-fold and a fourth gene, located just downstream of *ape0758*, *serC* which encodes a phosphoserine aminotransferase was upregulated by more than 2.5-fold due to HlyX.

Different terminal reductases transferring respiratory chain electrons to alternative electron acceptors other than oxygen were found to be strongly upregulated by HlyX. The three genes *dmsA*, *dmsB* and *dmsC* encoding the anaerobic dimethylsulfoxide reductase were found to be upregulated nearly 25-fold, 7.5-fold and 8.5-fold, respectively. The periplasmic nitrate reductase is encoded in *E. coli* by the *napFDAGHBC* operon. Homologues to all of these genes were found in *A. pleuropneumoniae* and were strongly upregulated due to HlyX (more the 5-fold each). The four genes encoding the proteins forming the nitrite reductase complex, *nrfABCD*, were identified to be also strongly upregulated by HlyX. The genes *ape1625* and *ape1626* share homology with *E. coli nrfF* and *nrfG* encoding for a heme lyase adding heme groups to NrfA; they were also identified to be upregulated by HlyX. The genes *torZ* and *torY* that are more then 10-fold upregulated by HlyX encode an additional reductase, the TMAO reductase. In contrast, three of four genes encoding the fumarate reductase enzyme complex, *frdABC*, are between 1.5 and 2-fold downregulated due to HlyX.

Several genes coding for enzymes which catalyze the oxidation of high energy substrates in order to transfer the electrons into the respiratory chain were identified as being HlyX regulated. Four genes (*hyaAB* and *hybAB*) encoding a Ni/Fe cofactor dependent hydrogenase were identified as being more then 10-fold upregulated due to HlyX; additionally *hyaD*, *hypB*, *hypD*, *hypE* and *hypF* involved in hydrogenase maturation were upregulated by HlyX above 2-fold, each. *E. coli* encodes at least three different hydrogenases. BLAST homology search revealed that *A. pleuropneumoniae* encodes only a single hydrogenase which is homologue to hydrogenase 2 of *E. coli* (*hyaAB* and *hybAB* of *A. pleuropneumoniae* are homologue to *hybABOC* of *E. coli*). A putative NAD(P)H oxidoreductase gene, *ape1539*, was also found to be about 12-fold upregulated by HlyX. Although the *glp* regulon repressor gene *glpR* was downregulated by HlyX the *glp* regulon was also downregulated in *A. pleuropneumoniae*. In *E. coli* it consists of the *glpABC* operon encoding the glycerol-3-phosphate dehydrogenase enzyme complex; in *A.*

pleuropneumoniae homologues to *glpAB* were identified, and they are downregulated by HlyX. Additionally, the *glpFK* and *glpTQ* operons were found to be downregulated by HlyX.

The gene *hcr* encoding a respiratory chain NADH reductase and *hcp* encoding the hybrid-cluster protein that is reduced due to oxidation of NADH were also found to be positively regulated by HlyX. The function of Hcr and Hcp are unknown (van den Berg et al., 2000).

The *adh2* gene of *A. pleuropneumoniae* shares homology to the *adhE* gene of *E. coli* that encodes for a protein with three catalytic functions, alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate formate–lyase deactivase. Adh2 might contribute to fermentation and was found in *A. pleuropneumoniae* as strongly upregulated by HlyX.

HlyX controls the expression of genes that encode proteins associated with iron acquisition in *A. pleuropneumoniae*. The genes *ape2089*, *ape2088*, *ape2087* and *ape2086* are annotated as “outer membrane receptor proteins, mostly iron transport” and were found to be upregulated more then 8-fold by HlyX. The *tonB* dependent transferrin binding protein encoding gene *tbpA* was upregulated about 2.5 fold. *A. pleuropneumoniae* has two genes with homology to *tonB* genes of other bacteria, *tonB1* and *tonB2*. Their transcription is upregulated 2.5-fold and 9-fold, respectively, due to HlyX. Further, two variants of the TonB interacting proteins ExbB and ExbD are expressed in *A. pleuropneumoniae*. The genes *expB* and *expB2* as well as *expD* and *expD2* were identified as strongly upregulated by HlyX. The ferric iron ABC transporter genes *ape0776* and *afuA_2* were also upregulated by HlyX whereas the *afuABC* genes were found to be downregulated by a factor of about 3 by HlyX.

Genes encoding different chaperone proteins including *hptG*, *dnaK*, *clpB*, *dnaJ*, *groEL*, and *groES* were found to be upregulated by HlyX.

The following ribosomal proteins were downregulated in *A. pleuropneumoniae* by HlyX: *rpsA*, *rplS*, *rpmL*, *rpsJ*, *rplI*, *rplX*, *rplO*, *rpsF*, *rpsM*, *rplW*, *rpsQ*, *rplC*, *rpsK*, *rplQ*, *rpsN*, *rplV*, *rpsD*, *rplR*, *rpsL*, *rplD*, *rpsH*, *rplT*, *rpsG*, *rplB*, *rplE*, *rplL*, *rpsC* and *rplP*.

The strongest downregulated gene (by a factor above 5) was identified as *sodA* encoding the manganese dependent superoxide dismutase which catalyzes the transformation of superoxide radicals into hydrogen peroxide. However, a cytochrome c peroxidase gene, *ccp*, that detoxifies hydrogen peroxide by transformation into water, was one of the strongest HlyX-upregulated genes (factor above 20).

HlyX regulated genes were analyzed using the “Pathway Tools Omics Viewer” provided on the biocyc homepage (http://biocyc.org). Using this software the *A. pleuropneumoniae* gene names were matched with metabolic pathways known for *E. coli*. Thereby a pathway map was created allowing an overview over metabolic pathways affected by HlyX. If several steps of a certain pathway were affected unanimously by HlyX the entire pathway appeared to be controlled by HlyX. This led to the identification of pathways that were up- and downregulated by HlyX (Fig. 17).

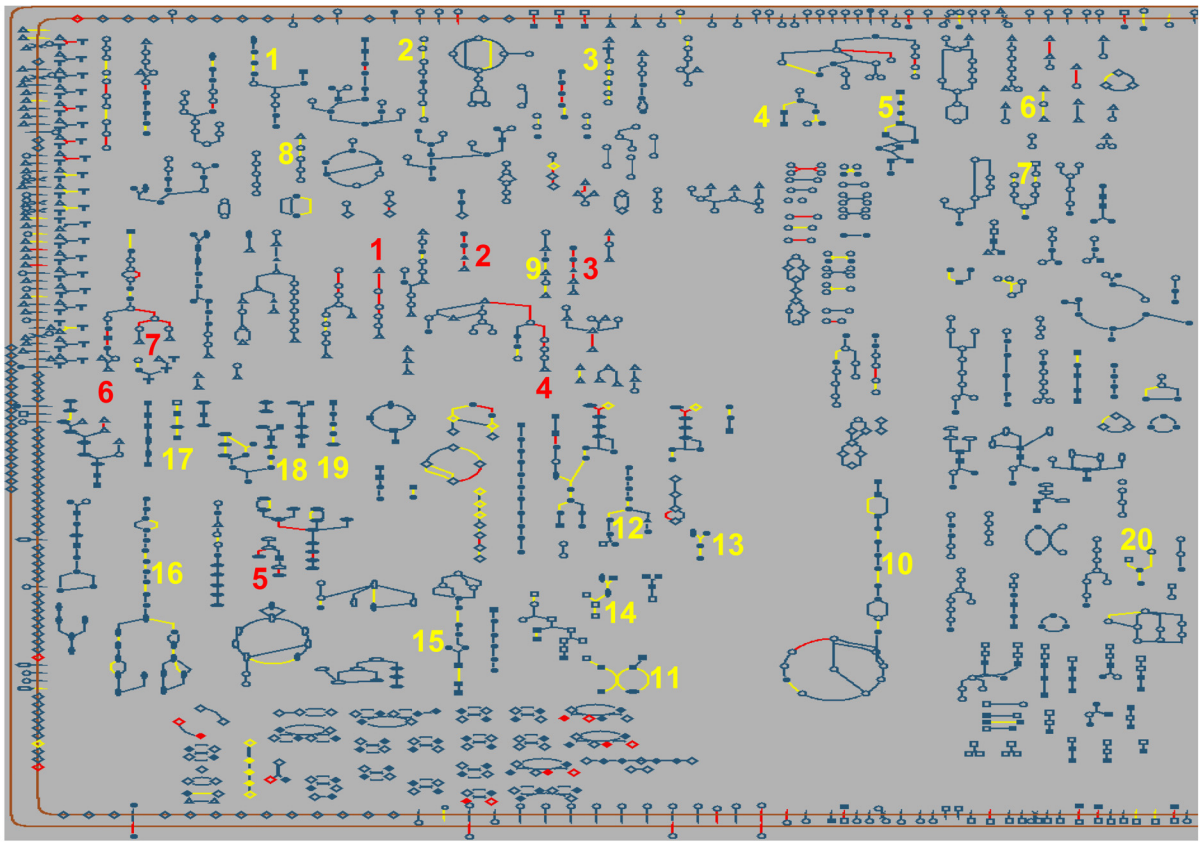


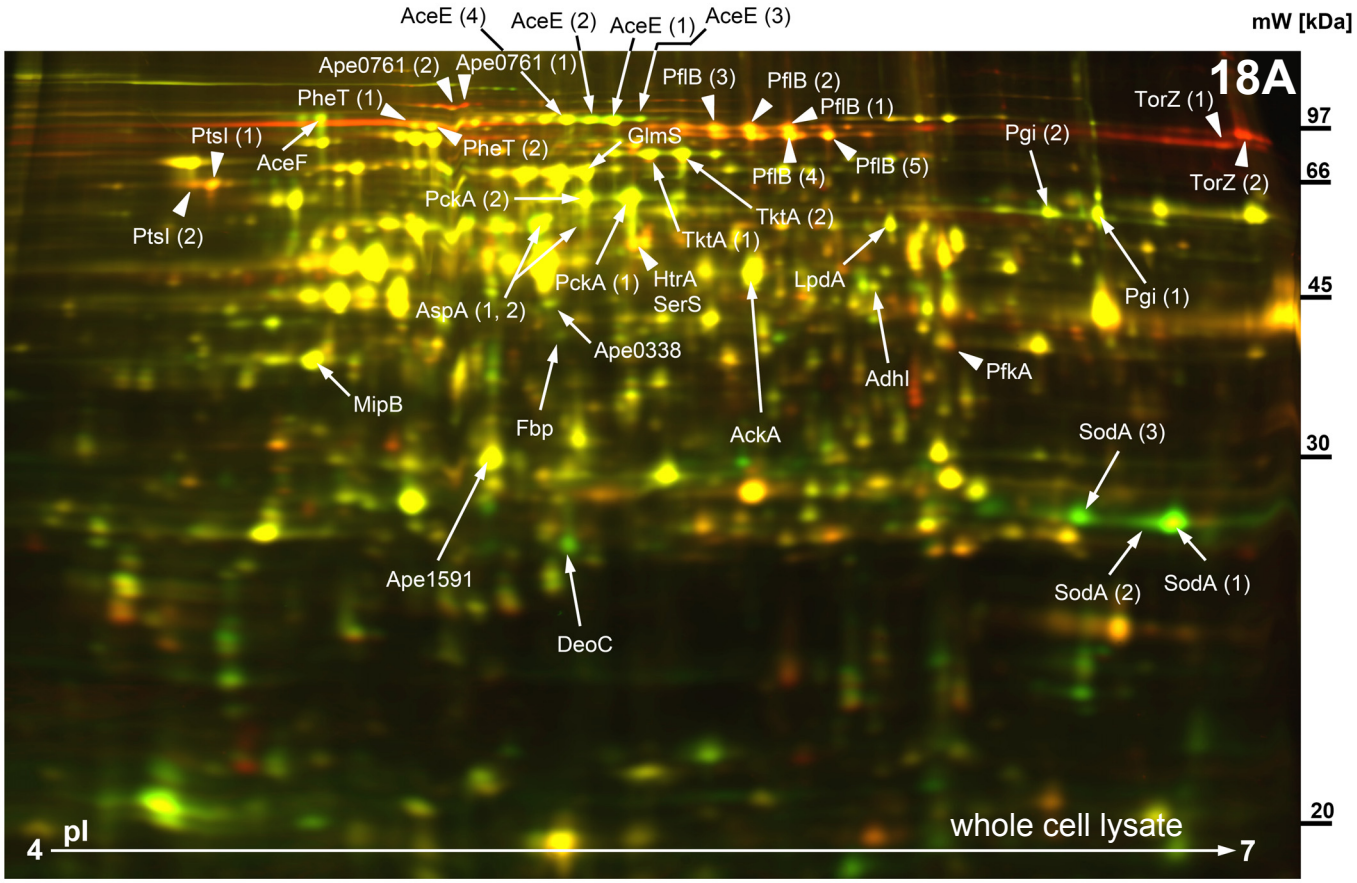
Fig. 17: Pathway map including HlyX regulated genes. *A. pleuropneumoniae* genes that were identified as HlyX regulated were matched into an *E. coli* pathway map using the "Pathway Tools Omics Viewer" (http://biocyc.org). Genes that were more then 1.5-fold downregulated by HlyX encode enzymes catalyzing reactions marked in yellow. Genes that were more then 1.5-fold upregulated by HlyX encode enzymes catalyzing reactions marked in red. If within a certain pathway several enzymes were affected by HlyX unanimously the respective pathway was analyzed (numbers).

Downregulated by HlyX (yellow numbers): 1. flavin biosynthesis; 2. menaquinone biosynthesis; 3. tetrapyrrole biosynthesis I; 4. superpathway of gluconate degradation; 5. superpathway pentose phosphate pathway; 6. proline degradation I; 7. superpathway of galacturonate and gluconate degradation; 8. biotin biosynthesis I; 9. proline biosynthesis I; 10. superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxalate bypass; 11. UDP-galactose biosynthesis; 12. phospholipid biosynthesis; 13. KDO transfer to lipid IV_A; 14. glycogen biosynthesis I (from ADP glucose); 15. gluconeogenesis; 16. purine nucleotides *de novo* biosynthesis; 17. colonic acid building blocks biosynthesis; 18. enterobacterial common antigen biosynthesis; 19. UDP-N-acetyl-D-glucosamine biosynthesis I; 20. glycerol degradation

Upregulated by HlyX (red numbers): 1. isoleucine biosynthesis from threonin; 2. serine biosynthesis; 3. glycine biosynthesis; 4. isoleucine biosynthesis from threonin; 5. salvage pathways of pyrimidine deoxyribonucleotides; 6. tryptophan biosynthesis; 7. phenylalanine and tyrosine biosynthesis

D 3.2 Global protein expression analysis of *A. pleuropneumoniae* Δ*hlyX*

2D DIGE and quantitative 1D PAGE were used for the identification of proteins that were regulated by HlyX of *A. pleuropneumoniae*. The respective proteins were identified by subsequent mass spectrometry. By applying different protein preparation methods to anaerobically grown *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX* the number of proteins identified by 2D DIGE analysis could be increased. 2D DIGE analyses were performed from i), ii) whole cell lysates (Fig. 18A, 18B), iii) inner and outer membrane-associated proteins (Fig. 18C), iv) outer membrane-associated proteins (Fig. 18D), and v) secreted proteins (Fig. 18E). Outer membrane proteins were analysed by quantitative 1D PAGE (Fig. 19). Expression of 492 proteins was found to be significantly regulated by HlyX comparing *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX* upon anaerobic growth (Table 7). Of 241 protein spots that were upregulated by HlyX, 75 were identified by mass spectrometry representing 21 different proteins (Table 7, 8). Of 251 spots that were found to be downregulated by HlyX, 79 could be identified by mass spectrometry representing 35 different proteins (Table 7, 9). The 154 protein spots identified in total represented 51 different proteins (5 proteins were identified either up- as well as downregulated by HlyX, dependent on the protein preparation method; Table 7). Additionally, five protein spots that were not found to be regulated statistically significant were analyzed by mass spectrometry (Table 10). 159 MS analyses were performed for the identification of HlyX regulated proteins; 104 of these analyses were done by Q-TOF MSMS (Appendix G 6.1), and 55 spots were identified by MALDI TOF MS (Appendix G 6.2).


18A
mW [kDa]
97
66
45
30
20
AceE (4)
AceE (2)
AceE (1)
AceE (3)
Ape0761 (2)
Ape0761 (1)
PflB (3)
PflB (2)
PflB (1)
PheT (1)
PheT (2)
PtsI (1)
AceF
PtsI (2)
GlmS
PckA (2)
TktA (2)
TktA (1)
PflB (4)
PflB (5)
Pgi (2)
TorZ (1)
TorZ (2)
Pgi (1)
PckA (1)
HtrA
SerS
AspA (1, 2)
LpdA
Ape0338
AdhI
PfkA
MipB
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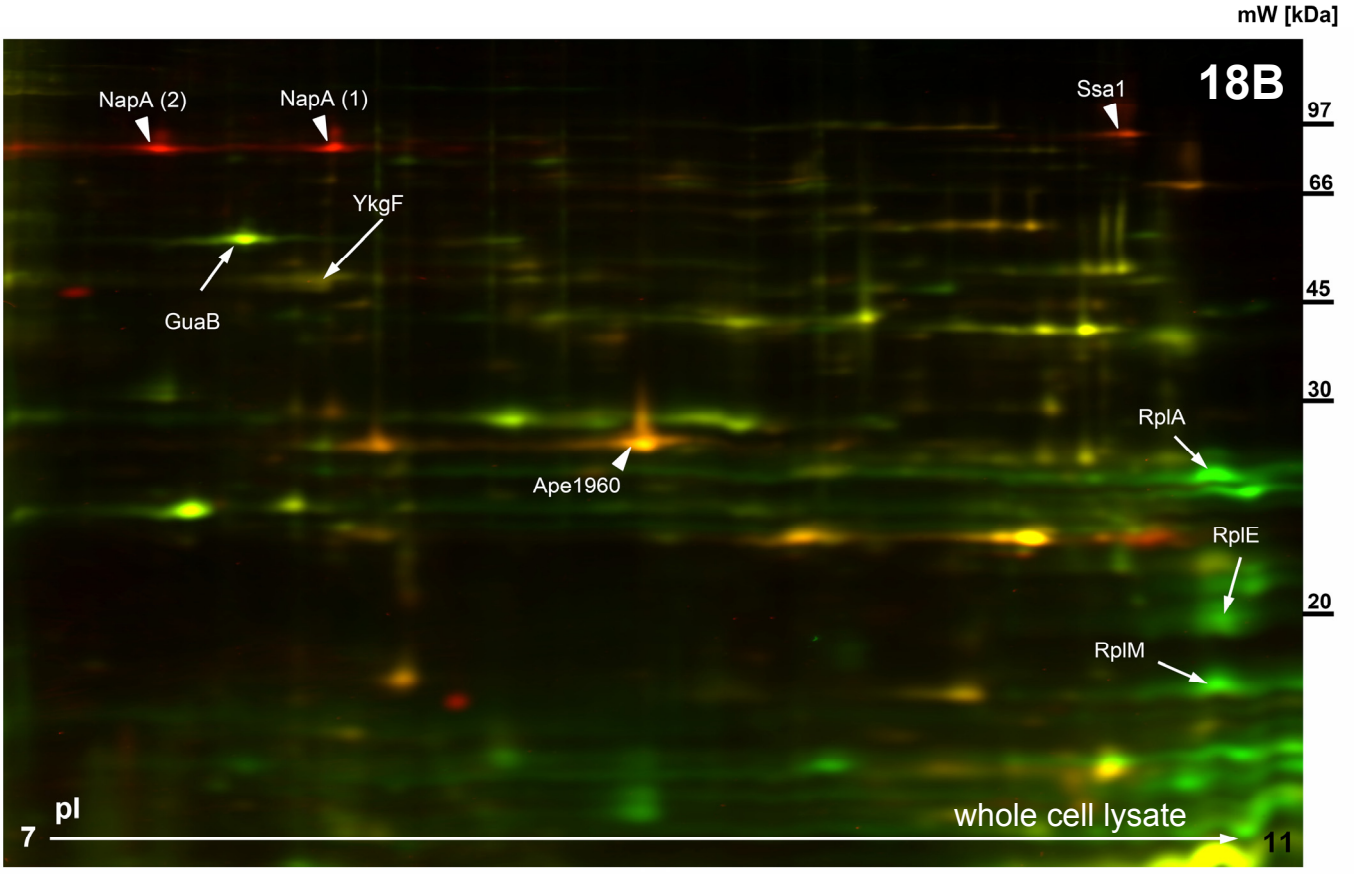
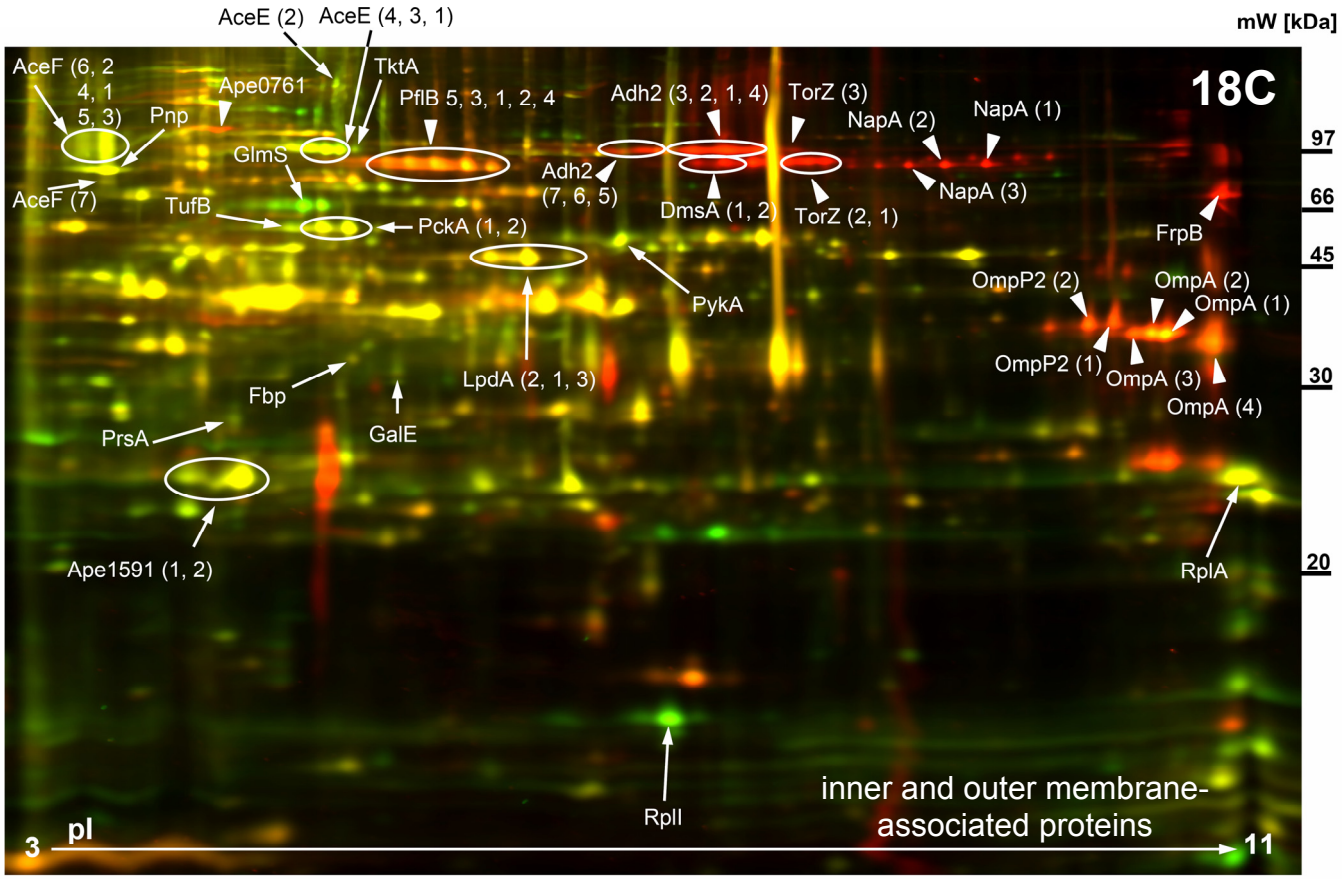
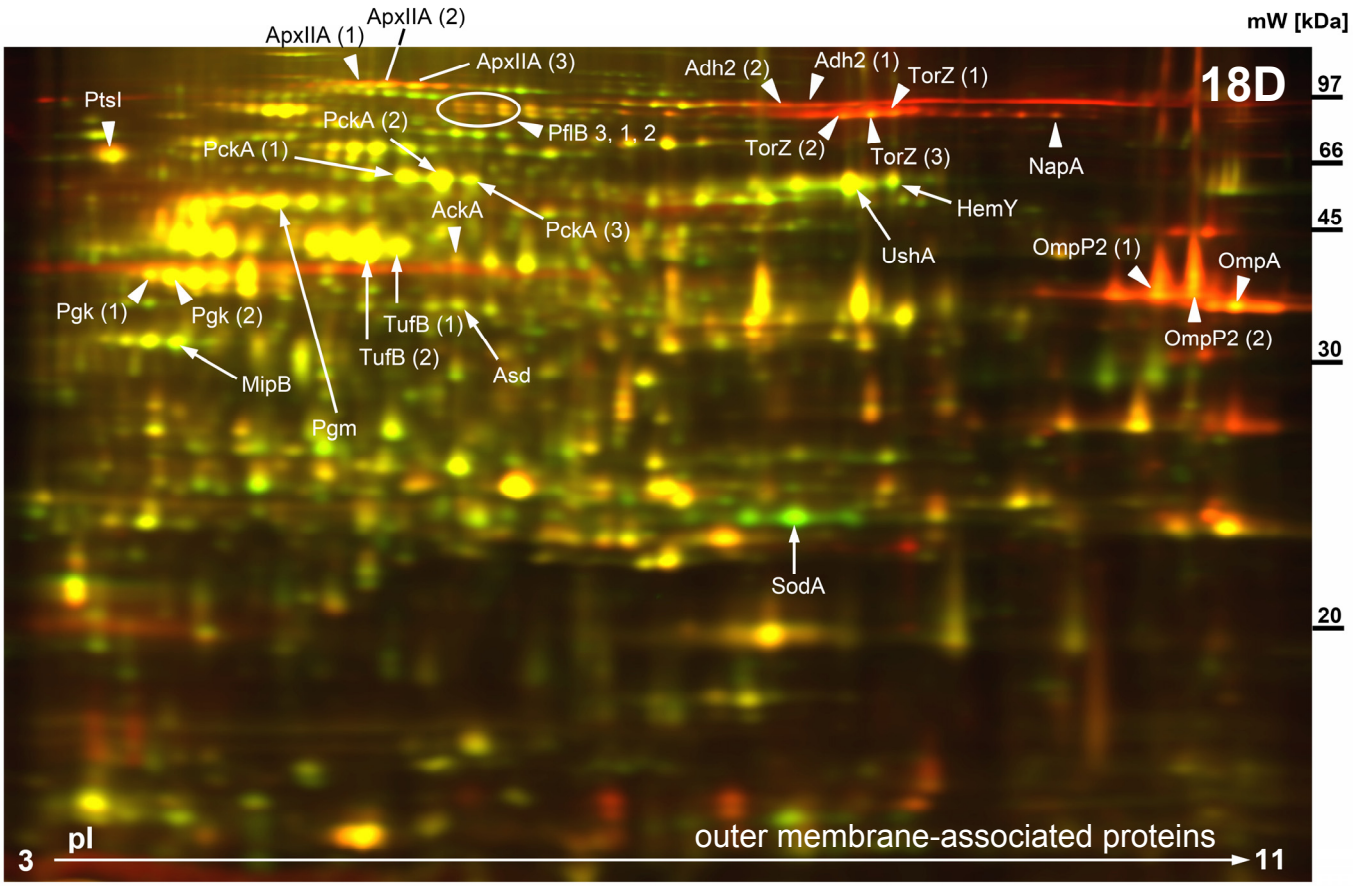
pI
4
7
whole cell lysate


18B
mW [kDa]
97
66
45
30
20
NapA (2)
NapA (1)
Ssa1
YkgF
GuaB
RplA
Ape1960
RplE
RplM
pI
7
11
whole cell lysate


mW [kDa]
18C
AceE (2)
AceE (4, 3, 1)
AceF (6, 2 4, 1 5, 3)
Pnp
Ape0761
TktA
PflB 5, 3, 1, 2, 4
Adh2 (3, 2, 1, 4)
TorZ (3)
NapA (2)
NapA (1)
GlmS
Adh2 (7, 6, 5)
NapA (3)
AceF (7)
TufB
PckA (1, 2)
DmsA (1, 2)
TorZ (2, 1)
FrpB
PykA
OmpP2 (2)
OmpA (2)
OmpA (1)
LpdA (2, 1, 3)
OmpP2 (1)
OmpA (3)
Fbp
OmpA (4)
PrsA
GalE
Ape1591 (1, 2)
RplA
RplI
inner and outer membrane-associated proteins
pI
3
11
97
66
45
30
20


mW [kDa]
18D
ApxIIA (1)
ApxIIA (2)
ApxIIA (3)
Adh2 (2)
Adh2 (1)
TorZ (1)
PtsI
PckA (2)
PflB 3, 1, 2
PckA (1)
TorZ (2)
TorZ (3)
NapA
HemY
AckA
PckA (3)
UshA
OmpP2 (1)
OmpA
Pgk (1)
Pgk (2)
TufB (1)
OmpP2 (2)
TufB (2)
Asd
MipB
Pgm
SodA
outer membrane-associated proteins
pI
3
11
97
66
45
30
20

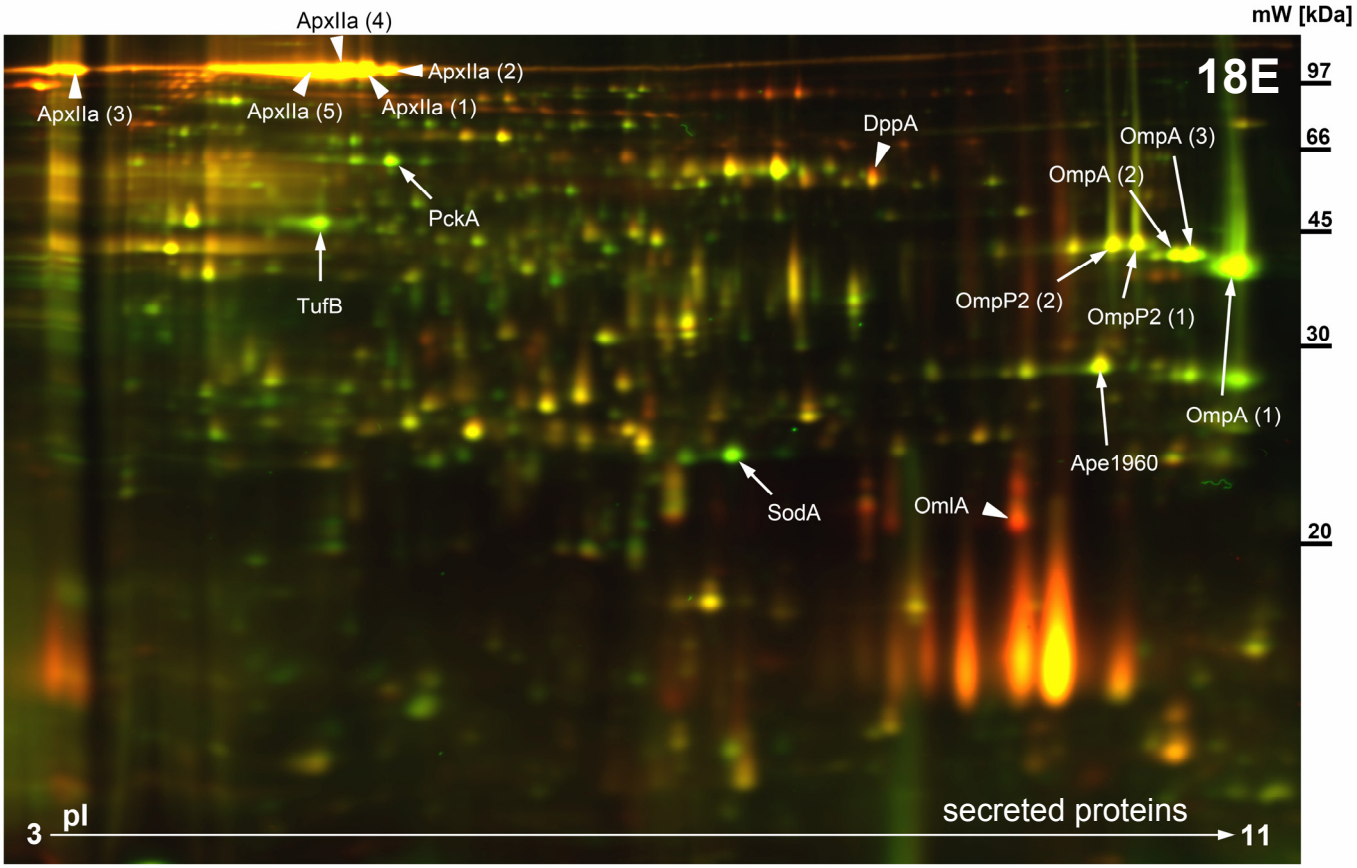


Fig. 18: 2D DIGE analysis of different protein preparations for comparison of protein expression between *A. pleuropneuomiae* wt (labelled with Cy5 [red]) and *A. pleuropneumoniae* Δ*hlyX* (labelled with Cy3 [green]).

Protein preparations and analyses were performed for *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX* as described in Fig. 12. Protein spots identified as significantly ($p < 0.05$) upregulated by HlyX are indicated by arrowheads. Spots identified as significantly downregulated ($p < 0.05$) by HlyX are indicated by arrows. Spots indicated by lines were not significantly regulated. Spots of interest were excised and analysed by mass spectrometry.

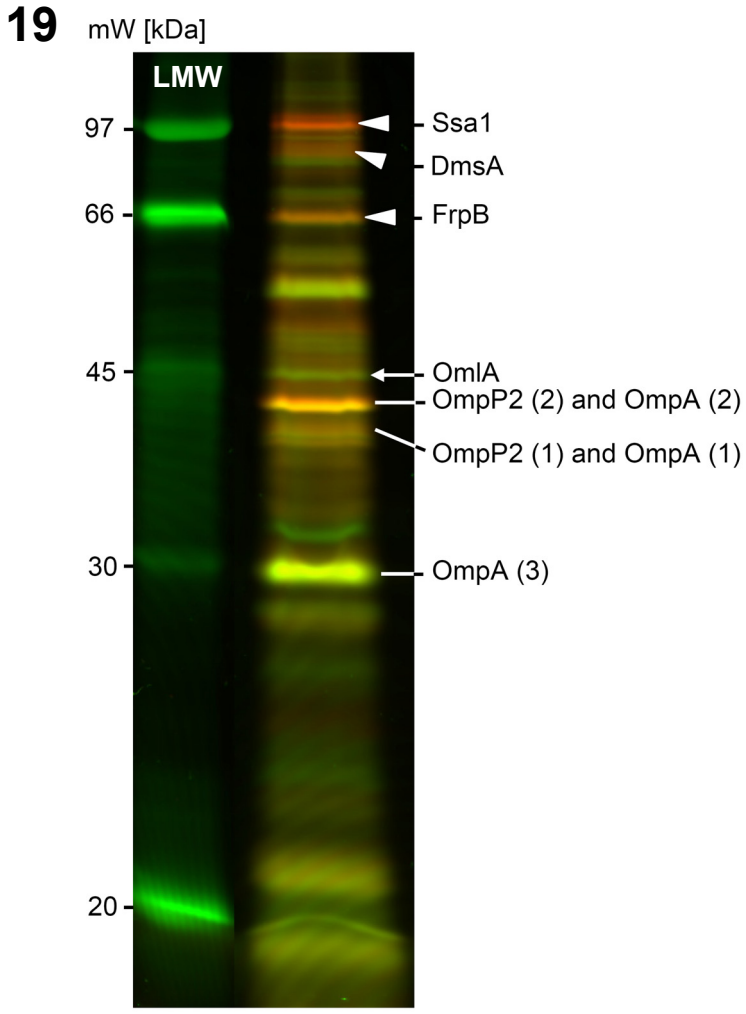


Fig. 19: **PAGE of an outer membrane protein preparation of *A. pleuropneumoniae* wt (labelled with Cy5 [red]) and *A. pleuropneumoniae* Δ*hlyX* (labelled with Cy3 [green]).** Protein preparations and analyses were performed for *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX* as described in Fig. 13. Protein bands identified as significantly ($p < 0.05$) upregulated by HlyX are indicated by arrowsheads. Band that were identified as significantly ($p < 0.05$) downregulated by HlyX are indicated by arrows. Band indicated by lines were not significantly regulated. Bands of interest were excised analysed by mass spectrometry.
LMW: Cy3 labelled low molecular weight marker (Amersham).

Table 7: Overview protein preparations, DIGE results and mass spectrometry results.

figure	protein preparation	IPG strip pI gradient	no. of differentially expressed spots [a]		no. of proteins identified by mass spectrometry		no. of different proteins identified	
			up in wt	up in Δ*hlyX*	up in wt	up in Δ*hlyX*	up in wt	up in Δ*hlyX*
18A	whole cell lysate	4 to 7	67	56	16	25	8	16
18B	whole cell lysate	7 to 11 NL[b]	19	15	4	5	3	5
18C	inner and outer membrane associated	3 to 11 NL[b]	76	110	28	28	9	15
18D	outer membrane-associated	3 to 11 NL[b]	63	52	17	11	10	8
18E	secreted	3 to 11 NL[b]	16	18	7	9	3	6
19	outer membranes	–	–	–	3	1	3	1
	total	–	**241**	**251**	**75**	**79**	**21**	**35**
					154		**51** [c]	

a) obtained by 2D DIGE as statistical significant (Student's T-test $p \leq 0.05$)

b) non linear

c) 21 different proteins upregulated plus 35 different proteins downregulated minus 5 proteins upregulated and downregulated by HlyX depending on the preparation method

Table 8: Proteins significantly upregulated by HlyX.

spot [a]	protein [b]	T-test [c]	ratio [d]	MS # [e]	gel # [f]
whole cell lysates (Fig. 18A)					
TorZ (1)	trimethylamine N oxide reductase precursor	0.0002	**26.6**	61	7
TorZ (2)	trimethylamine N oxide reductase precursor	0.005	**13.23**	41	3
Ape0761 (1)	putative methylation subunit type III restriction modification system	0.0072	**6.6**	209	6
Ape0761 (2)	putative methylation subunit type III restriction modification system	0.004	**4.49**	210	6
PflB (1)	formate acetyltransferase	0.001	**3.17**	17	3
PflB (2)	formate acetyltransferase	7.00E-05	**3.05**	15	3
PflB (3)	formate acetyltransferase	1.50E-05	**2.82**	18	3
PheT (1)	phenylalanyl tRNA synthetase beta chain	0.0015	**2.69**	26	3
PflB (4)	formate acetyltransferase	0.00027	**2.65**	22	3
PflB (5)	formate acetyltransferase	0.0054	**2.34**	21	3
PtsI (1)	phosphoenolpyruvate protein phosphotransferase	0.0021	**2.19**	27	3
PtsI (2)	phosphoenolpyruvate protein phosphotransferase	0.0021	**2.07**	218	6
PheT (2)	phenylalanyl tRNA synthetase beta chain	0.00093	**1.81**	25	3
HtrA	probable periplasmic serine protease do hhoA like precursor	0.005	**1.57**	34	3
SerS	seryl tRNA synthetase	0.005	**1.57**	35	3
PfkA	6 phosphofructokinase	0.024	**1.18**	68	3
whole cell lysates (Fig. 18B)					
NapA (1)	periplasmic nitrate reductase precursor	1.10E-05	**17.97**	175	9
NapA (2)	periplasmic nitrate reductase precursor	6.00E-05	**15.62**	168	10
Ssa1	serotype specific antigen 1 precursor	0.0048	**5.86**	171	10
Ape1960	hypothetical protein APL 1832	0.0028	**1.74**	177	10
inner and outer membrane-associated proteins (Fig. 18C)					
TorZ (1)	trimethylamine-N-oxide reductase precursor	7.00E-06	**22.76**	M58	11
TorZ (2)	trimethylamine-N-oxide reductase precursor	0.00027	**20.57**	M57	11

NapA (1)	periplasmic nitrate reductase precursor	0.00031	**17.29**	263	11
NapA (2)	periplasmic nitrate reductase precursor	0.00022	**16.25**	264	11
NapA (3)	periplasmic nitrate reductase precursor	5.80E-05	**15.23**	M59	11
DmsA (1)	anaerobic dimethyl sulfoxide reductase chain A precursor	4.90E-05	**14.17**	273	11
Adh2 (1)	aldehyde alcohol dehydrogenase 2	0.0011	**13.75**	271	11
Adh2 (2)	aldehyde-alcohol dehydrogenase 2	2.20E-05	**13.22**	M55	11
Adh2 (3)	aldehyde-alcohol dehydrogenase 2	7.60E-05	**11.65**	M54	11
TorZ (3)	trimethylamine-N-oxide reductase precursor	7.70E-06	**10.98**	M56	11
DmsA (2)	anaerobic dimethyl sulfoxide reductase chain A precursor	0.00034	**10.97**	274	11
Adh2 (4)	aldehyde alcohol dehydrogenase 2	0.00046	**9.98**	265	11
FrpB	iron regulated outer membrane protein B	0.0034	**8.8**	260	11
Adh2 (5)	aldehyde-alcohol dehydrogenase 2	0.017	**8.06**	M53	11
OmpA (1)	outer membrane protein P5 precursor	0.0035	**5.11**	258	11
OmpA (2)	outer membrane protein P5 precursor	0.0043	**4.77**	256	11
Ape0761	putative methylation subunit type III restriction modification system	7.20E-05	**4.6**	267	11
OmpA (3)	outer membrane protein P5 precursor	0.0041	**4.48**	255	11
OmpP2 (1)	outer membrane protein P2 precursor OMP P2	0.022	**4.25**	254	11
OmpP2 (2)	outer membrane protein P2 precursor OMP P2	0.013	**3.96**	253	11
OmpA (4)	outer membrane protein P5 precursor OMP P5	0.0082	**3.93**	261	11
PflB (1)	formate acetyltransferase	4.00E-06	**3.02**	M41	11
Adh2 (6)	aldehyde-alcohol dehydrogenase 2	0.0065	**2.98**	M52	11
PflB (2)	formate acetyltransferase	2.50E-05	**2.96**	M42	11
PflB (3)	formate acetyltransferase	1.00E-05	**2.88**	M40	11
PflB (4)	formate acetyltransferase	0.00061	**2.58**	287	11
Adh2 (7)	aldehyde-alcohol dehydrogenase 2	0.0027	**2.52**	M51	11
PflB (5)	formate acetyltransferase	6.40E-05	**2.31**	268	11
outer membrane-associated proteins (Fig. 18D)					
TorZ (1)	trimethylamine-N-oxide reductase precursor	0.00012	**17.3**	M90	13
NapA	periplasmic nitrate reductase precursor	0.0053	**13.34**	M91	13
TorZ (2)	trimethylamine-N-oxide reductase precursor	0.00017	**11.19**	M21	13
Adh2 (1)	aldehyde-alcohol dehydrogenase 2	0.0017	**10.8**	M88	13
TorZ (3)	trimethylamine-N-oxide reductase precursor	4.40E-05	**10.33**	M89	13
OmpA	outer membrane protein P5 precursor	0.0022	**10.18**	M29	13
Adh2 (2)	aldehyde-alcohol dehydrogenase 2	0.00016	**9.12**	M87	13
ApxIIA (1)	RTX-II toxin determinant A	0.047	**7.86**	M13	13
OmpP2 (1)	outer membrane protein P2 precursor OMP P2	0.0088	**5.73**	278	12
OmpP2 (2)	outer membrane protein P2 precursor OMP P2	0.013	**5.31**	279	12
PflB (1)	formate acetyltransferase	0.025	**2.35**	M85	13
PflB (2)	formate acetyltransferase	0.03	**2.3**	M86	13
PflB (3)	formate acetyltransferase	0.04	**2.26**	M84	13
Pgk (1)	phosphoglycerate kinase	1.50E-05	**1.95**	M99	13
AckA	acetate kinase	0.0053	**1.73**	235	13
PtsI	phosphoenolpyruvate protein phosphotransferase	0.032	**1.46**	M92	13
Pgk (2)	phosphoglycerate kinase	0.023	**1.37**	M100	13
secreted proteins (Fig. 18E)					
OmlA	outer membrane lipoprotein	0.013	**4.13**	131	14
ApxIIA (1)	RTX-II toxin determinant A	0.0033	**2.27**	M06	14
ApxIIA (2)	RTX-II toxin determinant A	0.021	**1.87**	M07	14
ApxIIA (3)	RTX II toxin determinant A	0.0033	**1.78**	154	14
ApxIIA (4)	RTX-II toxin determinant A	0.016	**1.75**	M05	14
DppA	periplasmic dipeptide transport protein	0.031	**1.62**	146	14
ApxIIA (5)	RTX-II toxin determinant A	0.035	**1.62**	M04	14

outer membrane proteins (Fig. 19)					
Ssa1	serotype specific antigen 1 precursor	0.00204504	**10.84**	90	17
FrpB	iron regulated outer membrane protein B	0.00998	**3.41**	75	16
DmsA	anaerobic dimethyl sulfoxide reductase chain A precursor	0.03276463	**2.29**	91	17

Table 9: Proteins significantly downregulated by HlyX.

spot [a]	protein [b]	T-test [c]	ratio [d]	MS # [e]	gel # [f]
whole cell lysates (Fig. 18A)					
SodA (1)	manganese superoxide dismutase	7.00E-06	**-6.4**	5	2
SodA (2)	manganese superoxide dismutase	6.50E-06	**-6.05**	66	7
SodA (3)	manganese superoxide dismutase	2.90E-05	**-5.34**	65	7
AceE (1)	pyruvate dehydrogenase E1 component	0.0008	**-2.31**	30	3
AceE (2)	pyruvate dehydrogenase E1 component	0.0023	**-2.04**	16	3
AspA (1)	aspartate ammonia lyase	0.013	**-1.98**	55	3
AspA (2)	aspartate ammonia lyase	0.0023	**-1.68**	62	5
PckA (1)	phosphoenolpyruvate carboxykinase ATP	0.00073	**-1.63**	33	3
Pgi (1)	glucose 6 phosphate isomerase	0.0018	**-1.6**	47	3
AceE (3)	pyruvate dehydrogenase E1 component	0.016	**-1.59**	M75	4
Ape0338	hypothetical protein APL 0319	0.001	**-1.57**	69	5
TktA (1)	transketolase 2	0.0049	**-1.57**	38	3
PckA (2)	phosphoenolpyruvate carboxykinase ATP	0.0063	**-1.54**	44	3
TktA (2)	transketolase 2	0.0049	**-1.5**	46	3
GlmS	glucosamine fructose 6 phosphate aminotransferase isomerizing	0.027	**-1.5**	49	3
AceF	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	0.017	**-1.48**	23	3
Pgi (2)	glucose 6 phosphate isomerase	0.0058	**-1.47**	43	3
LpdA	dihydrolipoyl dehydrogenase	0.02	**-1.44**	13	2
MipB	transaldolase	0.011	**-1.33**	71	3
AceE (4)	pyruvate dehydrogenase E1 component	0.023	**-1.33**	19	3
Fbp	fructose 1 6 bisphosphatase	0.026	**-1.33**	59	5
DeoC	deoxyribose phosphate aldolase	0.03	**-1.32**	215	6
AckA	acetate kinase	0.028	**-1.31**	53	3
Ape1591	hybrid peroxiredoxin HyPrx5	0.022	**-1.3**	72	3
AdhI	Alcohol dehydrogenase 1	0.03	**-1.25**	12	2
whole cell lysates (Fig. 18B)					
RplA	50S ribosomal protein L1	0.0057	**-14.44**	195	10
RplE	50S ribosomal protein L5	0.013	**-13.72**	203	10
RplM	50S ribosomal protein L13	0.019	**-9.64**	204	10
YkgF	putative electron transport protein	0.048	**-1.71**	174	9
GuaB	inosine 5 monophosphate dehydrogenase	0.01	**-1.57**	169	10
inner and outer membrane-associated proteins (Fig. 18C)					
RplI	50S ribosomal protein L9	0.0021	**-8.53**	269	11
RplA	50S ribosomal protein L1	0.012	**-2.95**	M50	11
AceF (1)	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)	0.00018	**-2.68**	M33	11
AceF (2)	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)	0.0015	**-2.65**	M32	11
AceF (3)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	0.00092	**-2.62**	237	11
AceF (4)	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)	0.00079	**-2.5**	M31	11
AceF (5)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	0.0019	**-2.47**	236	11

AceF (6)	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)	0.0019	**-2.45**	M30	11
AceE (1)	pyruvate dehydrogenase E1 component	3.60E-05	**-2.25**	M39	11
PykA	pyruvate kinase	0.00016	**-2.24**	262	11
AceF (7)	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2	0.026	**-2.18**	283	11
GlmS	glucosamine fructose 6 phosphate aminotransferase isomerizing	0.043	**-1.97**	249	11
TktA	Transketolase 2	7.00E-05	**-1.9**	M36	11
AceE (2)	pyruvate dehydrogenase E1 component	0.033	**-1.89**	277	11
AceE (3)	pyruvate dehydrogenase E1 component	0.00042	**-1.88**	M38	11
Fbp	fructose 1 6 bisphosphatase	0.0014	**-1.88**	266	11
Pnp	polyribonucleotide nucleotidyltransferase	0.03	**-1.87**	238	11
TufB	elongation factor Tu	0.00054	**-1.86**	248	11
PckA (1)	phosphoenolpyruvate carboxykinase ATP	0.0018	**-1.78**	252	11
LpdA (1)	dihydrolipoyl dehydrogenase	0.024	**-1.76**	M45	11
Ape1591 (1)	hybrid peroxiredoxin HyPrx5	0.0062	**-1.7**	242	11
LpdA (2)	dihydrolipoyl dehydrogenase	0.039	**-1.67**	M44	11
Ape1591 (2)	hybrid peroxiredoxin HyPrx5	0.0056	**-1.65**	243	11
PckA (2)	phosphoenolpyruvate carboxykinase (ATP)	0.0012	**-1.64**	M35	11
LpdA (3)	dihydrolipoyl dehydrogenase	0.0029	**-1.55**	291	11
PrsA	ribose phosphate pyrophosphokinase	0.035	**-1.5**	245	11
AceE (4)	pyruvate dehydrogenase E1 component	0.011	**-1.47**	M37	11
GalE	UDP glucose 4 epimerase Mannheimia haemolytica	0.011	**-1.34**	295	11
outer membrane-associated proteins (Fig. 18D)					
SodA	manganese superoxide dismutase	0.002	**-4.8**	M103	13
UshA	UshA precursor	0.023	**-2.02**	M105	13
HemY	HemY-like protein	0.02	**-1.99**	M104	13
MipB	Transaldolase	0.0068	**-1.75**	M102	13
TufB (1)	elongation factor Tu	0.0019	**-1.69**	M96	13
PckA (1)	phosphoenolpyruvate carboxykinase (ATP)	0.006	**-1.67**	M18	13
TufB (2)	elongation factor Tu	0.0007	**-1.65**	M95	13
PckA (2)	phosphoenolpyruvate carboxykinase ATP	0.013	**-1.65**	M82	13
PckA (3)	phosphoenolpyruvate carboxykinase ATP	0.0066	**-1.51**	M83	13
Asd	aspartate-semialdehyde dehydrogenase	0.0051	**-1.44**	M101	13
Pgm	phosphoglucomutase/phosphomannomutase	0.047	**-1.4**	M93	13
secreted proteins (Fig. 18E)					
SodA	manganese superoxide dismutase	0.00013	**-3.44**	138	14
OmpA (1)	outer membrane protein P5 precursor OMP P5	0.0009	**-2.71**	153	14
OmpA (2)	outer membrane protein P5 precursor	0.0027	**-2.59**	161	14
OmpA (3)	outer membrane protein P5 precursor	0.0011	**-2.46**	151	14
OmpP2 (1)	outer membrane protein P2 precursor OMP P2	0.036	**-1.96**	150	14
OmpP2 (2)	outer membrane protein P2 precursor OMP P2	0.038	**-1.93**	149	14
PckA	phosphoenolpyruvate carboxykinase (ATP)	0.05	**-1.7**	M08	14
TufB	elongation factor Tu	0.039	**-1.6**	148	14
Ape1960	hypothetical protein APL 1832	0.016	**-1.46**	129	14
outer membrane proteins (Fig. 19)					
OmlA	outer membrane lipoprotein	0.0048	**-2.89**	95	17

Table 10: Selected proteins not significantly up- or downregulated by HlyX.

spot [a]	protein [b]	T-test [c]	ratio [d]	MS # [e]	gel # [f]
outer membrane-associated proteins (Fig. 18D)					
ApxIIA (2)	RTX II toxin determinant A	0.086	**8.85**	230	13
ApxIIA (3)	RTX II toxin determinant A	0.087	**10.07**	281	13
outer membrane proteins (Fig. 19)					
OmpA (1)	outer membrane protein P5 precursor OMP P5	0.423	**1.52**	92	17
OmpA (1)	outer membrane protein P5 precursor	0.423	**1.52**	93	17
OmpP2 (1)	outer membrane protein P2 precursor OMP P2	0.423	**1.52**	94	17
OmpP2 (2)	outer membrane protein P2 precursor OMP P2	0.495	**1.31**	73	15
OmpA (2)	outer membrane protein P5 precursor OMP P5	0.495	**1.31**	82	16
OmpA (2)	outer membrane protein P5 precursor	0.495	**1.31**	84	16
OmpA (3)	outer membrane protein P5 precursor	0.582	**1.12**	79	16
[OmpA (3)]	outer membrane protein P5 precursor OMP P5	0.582	**1.12**	80	16

a) name of protein spot on gel (Fig. 18 A-E, 19)

b) result of mass spectrometry analysis.

c) statistical analysis using unpaired Student's T-test

d) ratio between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX*. Positive values indicate upregulation by HlyX, negative values indicate downregulation by HlyX.

e) number of the mass spectrometric analysis (Appendix G 6) that was performed for the identification of the protein of interest from the respective preparative gel [f]. Simple numbers indicate analysis by Q-TOF MSMS; numbers beginning with the capital letter M indicate analysis by MALDI-TOF MS.

f) this number indicates the preparative gel (Appendix G 5) from which the respective protein spot was obtained.

D 3.2.1 Analysis of differentially expressed proteins

Enzymes catalyzing the transfer of respiratory chain electrons to substrates different than oxygen were strongly upregulated anaerobically due to HlyX in *A. pleuropneumoniae*. The TMAO reductase (TorZ) was identified by 2D DIGE of whole cell lysates, inner and outer membrane-associated proteins and outer membrane-associated proteins (Fig. 18A, 18C, 18D) as upregulated more than 20-fold by HlyX. 2D DIGE analyses of these gels also revealed the periplasmic nitrate reductase subunit NapA as above 10-fold upregulated by HlyX. The anaerobic DMSO reductase subunit DmsA was identified on gels of inner and outer membrane- associated proteins (Fig. 18C) as upregulated more than 10-fold by HlyX, and quantitative 1D PAGE of outer membrane proteins (Fig. 19) showed a more than 2-fold upregulation.

The aldehyde alcohol dehydrogenase 2 (Adh2), an enzyme possibly being involved in fermentation was identified by 2D DIGE of inner and outer membrane-associated proteins (Fig. 18C) and outer membrane-associated proteins (Fig. 18D) as more than 10-fold

upregulated by HlyX. Additionally the formate acetyltransferase which converts pyruvate into formate and acetyl-CoA was identified as more than 3-fold upregulated by HlyX.

The putative methylation subunit of a type III restriction modification system (Ape0761) was also identified by 2D DIGE (Fig. 18A, 18C) as more than 6-fold upregulated by HlyX. Furthermore the serotype specific antigen 1 precursor that is a homologue to the autotransporter serine protease of *A. pleuropneumoniae* serotype 1 strain 4047 could be identified on gels of whole cell lysates (Fig. 18B) as well as on outer membrane protein gels (Fig. 19) as upregulated by HlyX.

2D DIGE analysis of whole cell lysates (Fig. 18A), outer membrane-associated proteins (Fig. 18D), and secreted proteins (Fig. 18E) revealed the manganese superoxide dismutase as the protein strongest downregulated by HlyX. The pyruvate dehydrogenase enzyme complex comprising AceE, AceF and LpdA and catalyzing the formation of the citric acid cycle precursor acetyl-CoA was also found to be downregulated by HlyX (Fig. 18A, 18C). The fructose-1,6-bisphosphatase (Fbp) and the phosphoenolpyruvate carboxykinase (PckA), both catalyzing essential steps of gluconeogenesis, were identified as slightly downregulated by HlyX (Fig. 18A, 18C, 18D, 18E).

D 3.2.2 Comparison between detergent and non detergent-dependent protein preparations

The quantification of differences in protein expression between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX* by 2D DIGE analyses showed mostly corresponding results for different methods of protein preparation. However, some proteins could be obtained from *A. pleuropneumoniae* wt in considerably higher yields when detergent was used during protein preparation. Thus, these proteins became enriched during preparation of inner and outer membrane-associated proteins (Fig. 18C) and outer membrane-associated proteins (Fig. 18D).

The iron regulated outer membrane protein B (FrpB) was identified as 8.8-fold upregulated due to HlyX on gels of inner and outer membrane-associated proteins (Fig 18C) compared to a 3.4-fold upregulation by HlyX obtained upon analysis of gels of outer membrane proteins (Fig. 19). Particularly striking was the detergent- based enrichment of the ApxIIA toxin from the wt strain compared to the *hlyX* deletion mutant. Thus, 2D DIGE analysis of outer membrane-associated proteins (Fig. 18D) revealed the ApxIIA toxin as being 7.9-fold upregulated by HlyX whereas analysis of secreted proteins (Fig. 18E) showed only a 1.6 to 2.3-fold upregulation by HlyX. The outer membrane proteins OmpA and OmpP2 were identified by analysis of inner and outer membrane-associated proteins (Fig 18C). On

gels of outer membrane-associated proteins (Fig. 18D) OmpA was found to be more than 10-fold upregulated by HlyX, and OmpP2 was found as more than 5-fold upregulated. However, 2D DIGE analysis of the non detergent-based preparation of secreted proteins (Fig. 18D) revealed OmpA and OmpP2 as being about 2-fold downregulated by HlyX. A quantitative analysis of outer membrane proteins showed only a slight (up to 1.5-fold) upregulation by HlyX which was statistically not significant.

D 3.2.3 Comparison of microarray and 2D DIGE analyses

The 2D DIGE analysis of protein expression essentially verified the results obtained by microarray analysis gene of expression (Fig. 20).

The transcripts of 19 different proteins that were identified as 65 different spots upregulated by HlyX were additionally identified by microarray analysis. For 15 of these proteins (55 spots) the transcript was also upregulated by HlyX whereas for four proteins (10 spots) the transcript was downregulated by HlyX (Fig. 20). The difference between proteomic and microarray analysis was most obvious for the ApxIIA toxin. Proteomic analysis of the detergent-based preparation of inner and outer membrane-associated proteins (Fig 18C) revealed a spot of the ApxIIA as 7.8-fold upregulated by HlyX, proteomic analysis of secreted proteins (Fig 18D) showed a upregulation of 5 spots of about 2-fold by HlyX whereas the respective transcript was identified to be 2-fold downregulated by HlyX.

For 60 of 67 protein spots resembling 24 different proteins that were identified by the proteomic approach as being downregulated by HlyX the respective transcript was also downregulated by HlyX. Seven protein spots that resemble six different proteins were identified by 2D DIGE as being downregulated by HlyX whereas their transcripts were found to be upregulated by HlyX. The outer membrane lipoprotein (OmlA) was identified by proteomic analysis of outer membrane proteins (Fig 19) as being 2.9-fold downregulated by HlyX whereas expression of the respective transcript was increased 1.5-fold. However, 2D DIGE analysis of secreted proteins (Fig 18D) revealed a 4.1-fold upregulation of OmlA by HlyX.

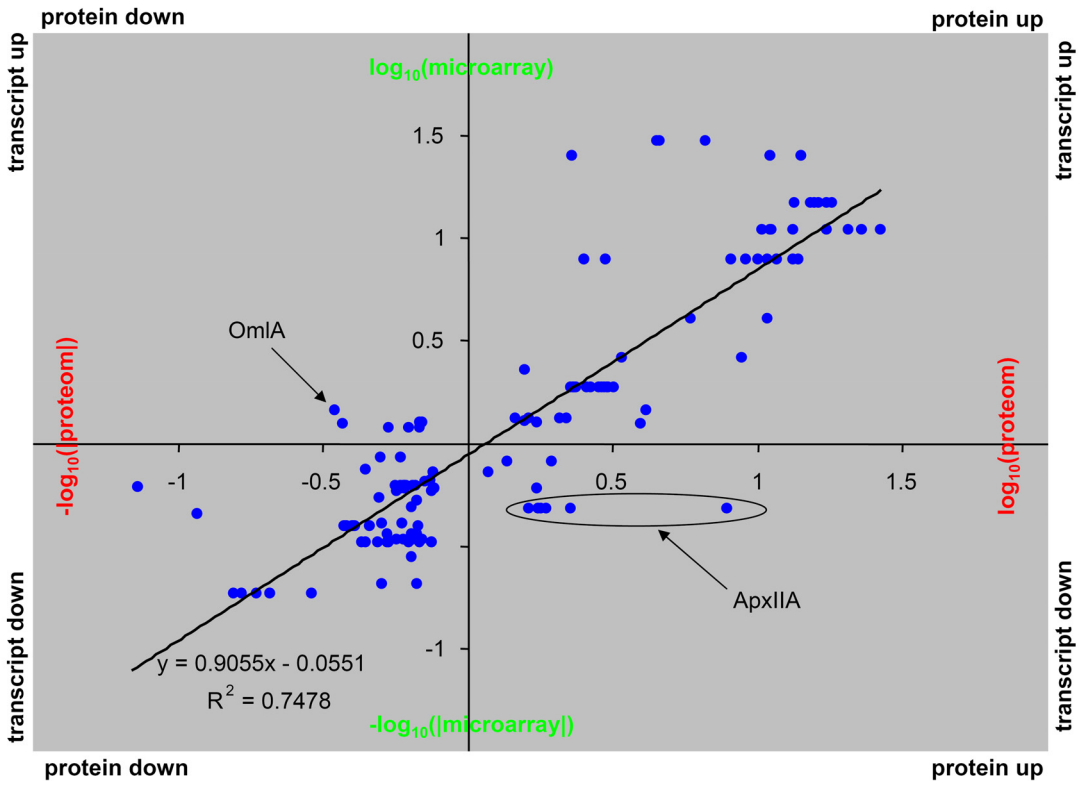


Fig. 20: Comparison of transcript (microarray) and protein (2D DIGE, quantitative PAGE) ratios between anaerobically grown *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX*. Transcripts as well as proteins that were identified to be upregulated due to HlyX received a positive value representing the factor of upregulation (Appendix G 3, Table 8). The $\log_{10}$ of these factors is represented by positive values on the y-axis for transcripts and positive values on the x-axis for proteins. Transcripts and proteins that were downregulated due to HlyX are indicated by a negative number representing the factor of downregulation (Appendix G 4, Table 9). The negative $\log_{10}$ of the absolute value is shown as negative values on the y-axis for transcripts and as negative values of the x-axis for proteins. The line of best fit was calculated showing a slope close to one. Proteins that deviated strongly from the line of best fit are indicated by arrows.

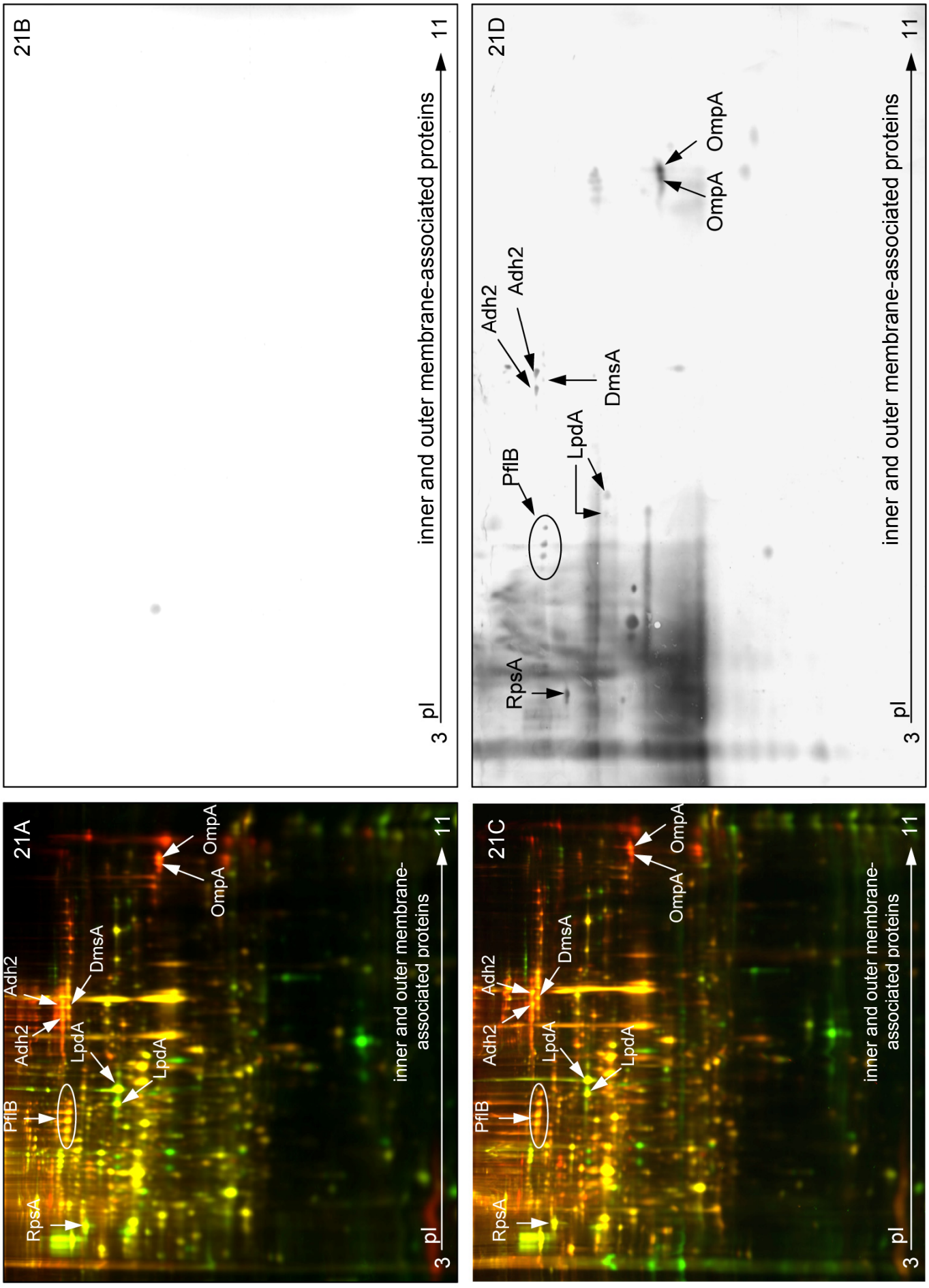
D 4 Identification of immunogenic proteins of *A. pleuropneumoniae*

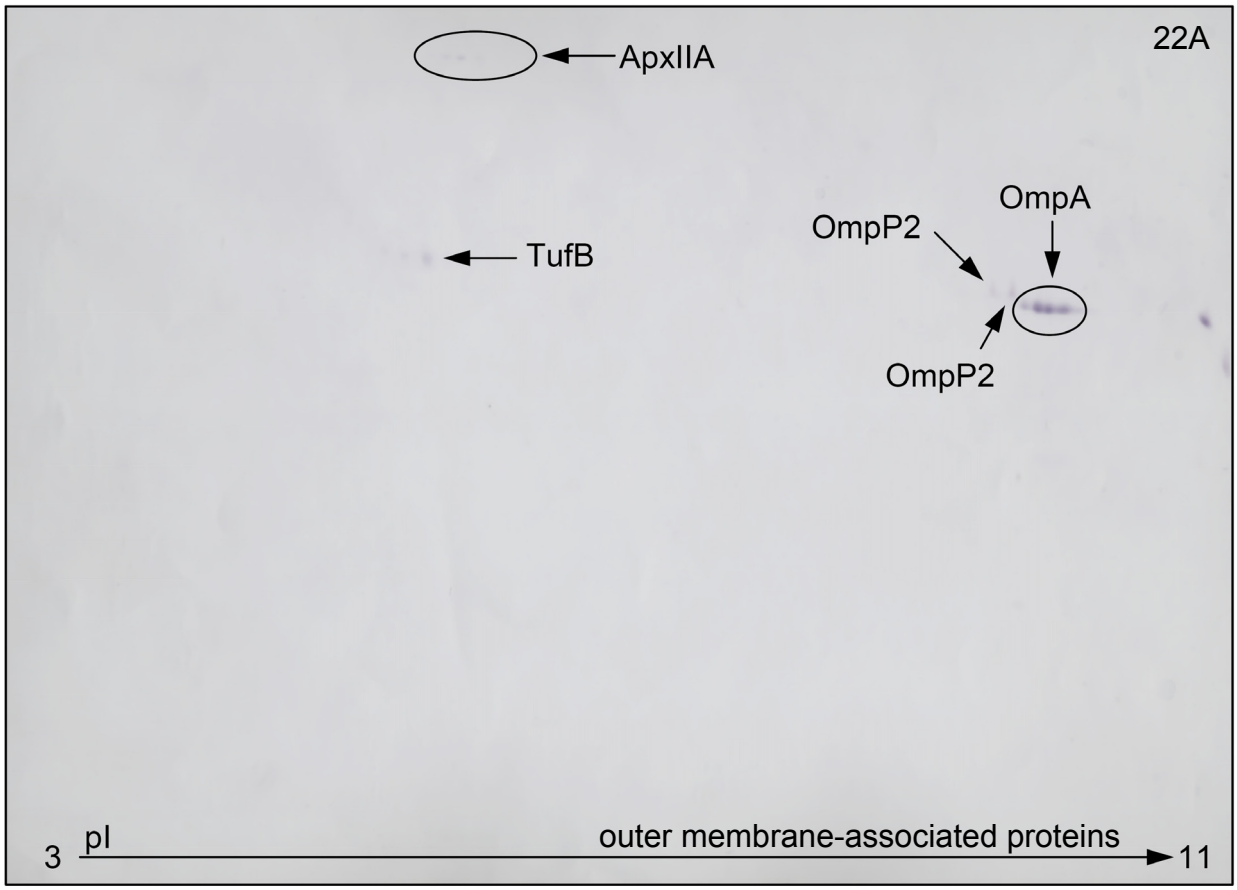
In order to identify immunogenic proteins of *A. pleuropneumoniae* different protein preparations were performed with *A. pleuropneumoniae* wt grown under anaerobic conditions. Inner and outer membrane-associated proteins (Fig. 21), outer membrane-associated proteins (Fig. 22), and secreted proteins (Fig. 23) were separated by two dimensional gel electrophoresis. Outer membrane proteins (Fig. 24) were separated by one dimensional PAGE. Then the proteins were blotted and incubated with preimmune sera obtained from pigs before experimental infection with *A. pleuropneumoniae* wt. These pigs were tested immunological for absence of antibodies against *A. pleuropneumoniae*. Proteins that were detected with these sera define the experimental background. Convalescent sera from pigs 21 days post infection with *A. pleuropneumoniae* wt contained antibodies that were developed against immunogenic proteins during the course of infection. Proteins that were detected exclusively by convalescent sera are immunogenic. By comparing the pattern of immunostained proteins on the blotting membrane with the pattern of stained proteins in gels, spots of interest were identified, and then excised from a preparative gel followed by mass spectrometry identification.

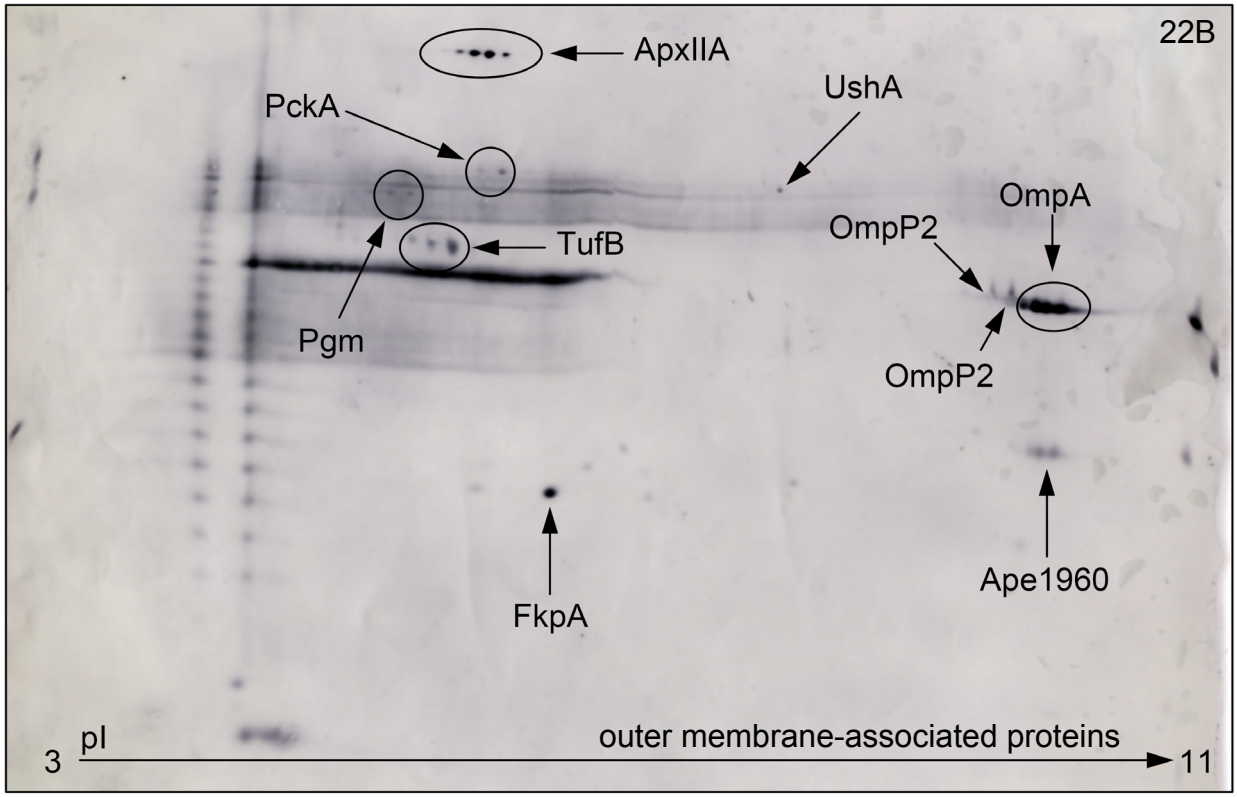
Altogether 16 different proteins of *A. pleuropneumoniae* were identified as being immunogenic from the different protein preparations (Table 11).

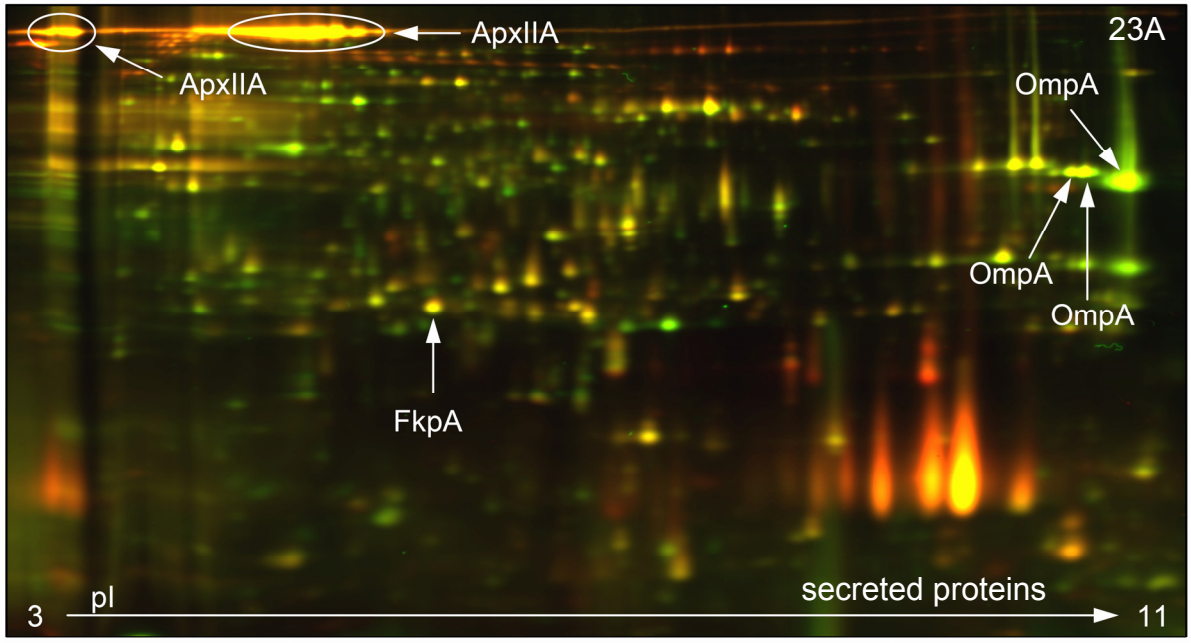
As strongly immunogenic proteins the ApxIIA toxin and the outer membrane proteins OmpA and OmpP2 were identified. The secreted ApxIIA toxin appeared as a horizontal sequence of spots on 2D gels due to slight differences in their pI values. All of these spots were identified from gels of outer membrane-associated proteins and secreted proteins (Fig. 22, 23). The outer membrane proteins OmpA and OmpP2 were identified on several gels (Fig. 21, 22, 23, 24) as targets for antibodies developed upon infection with *A. pleuropneumoniae*. In addition, proteins that are normally located within the bacterial cell like the 30S ribosomal protein S1 (RpsA) or the elongation factor Tu (TufB) caused the formation of antibodies in infected pigs.

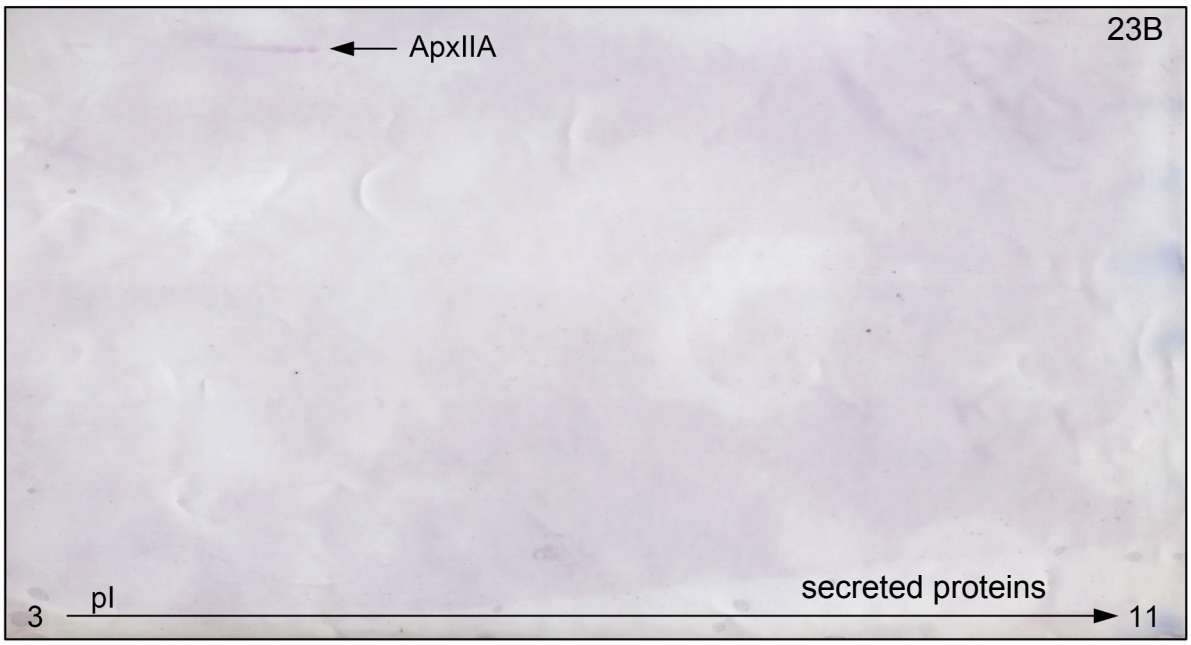
In the acidic pH range from < 3 up to about 5 on all 2D gels a ladder of horizontal lines appeared that was strongly immunogenic and showed almost now cross reaction with sera from pigs before infection. The same sequence of bands could be observed on the 1D gel, also. These bands did not appear on comparative gels stained with Coomassie blue and were not found as labelled with CyDyes™ before blotting. Therefore it is likely that this immunogenic material is not protein but rather lipopolysaccharide.

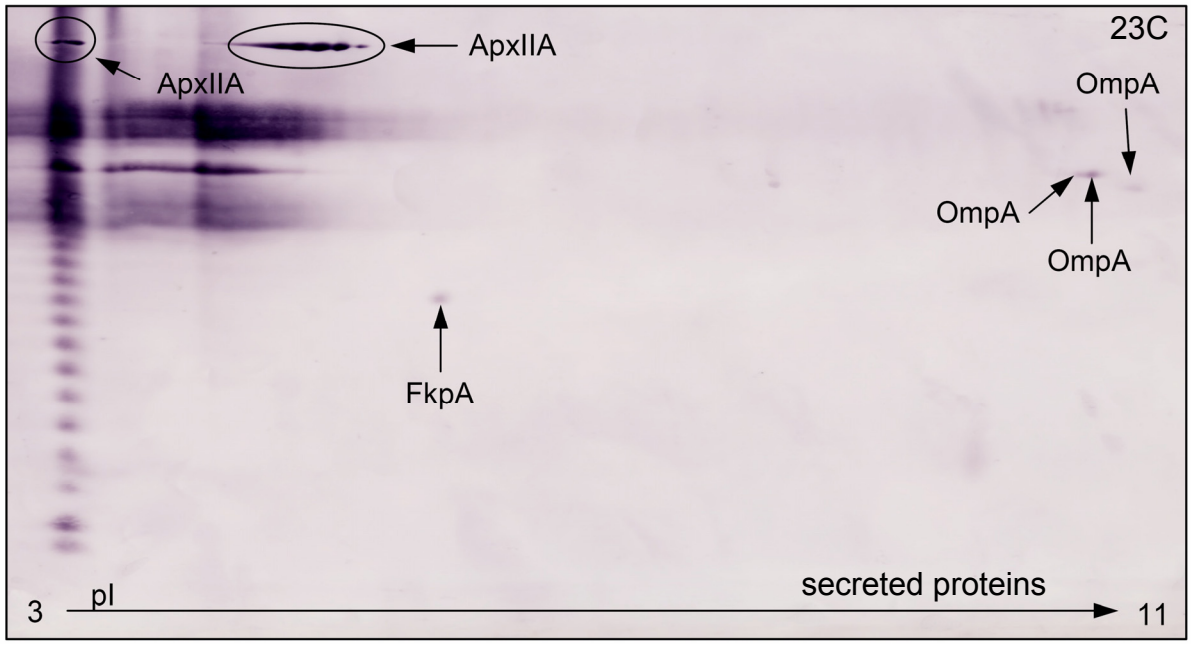

21A
RpsA
PflB
Adh2
Adh2
LpdA
DmsA
LpdA
OmpA
OmpA
inner and outer membrane-associated proteins
3
pl
11
21B
inner and outer membrane-associated proteins
3
pl
11
21C
RpsA
PflB
Adh2
Adh2
LpdA
DmsA
LpdA
OmpA
OmpA
inner and outer membrane-associated proteins
3
pl
11
21D
RpsA
PflB
Adh2
Adh2
LpdA
DmsA
OmpA
OmpA
inner and outer membrane-associated proteins
3
pl
11


22A
ApxIIA
TufB
OmpA
OmpP2
OmpP2
3
pI
outer membrane-associated proteins
11

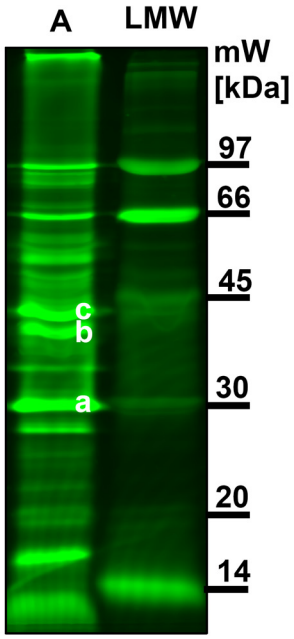

22B
ApxIIA
PckA
UshA
TufB
OmpA
OmpP2
Pgm
OmpP2
FkpA
Ape1960
3
pI
outer membrane-associated proteins
11


23A
ApxIIA
ApxIIA
OmpA
OmpA
OmpA
FkpA
3
pI
secreted proteins
11

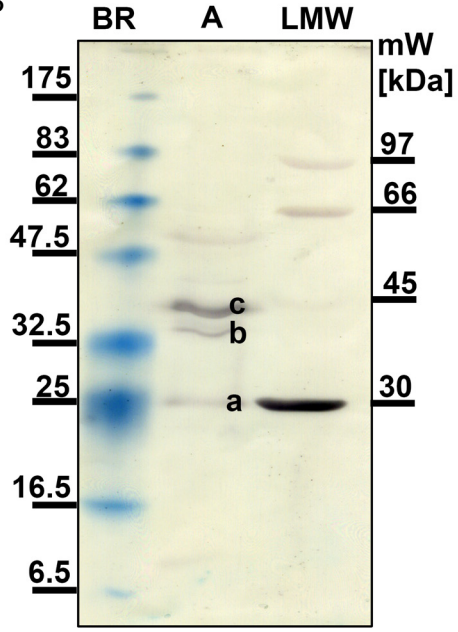

23B
ApxIIA
3
pI
secreted proteins
11


23C
ApxIIA
ApxIIA
OmpA
OmpA
OmpA
FkpA
3
pI
secreted proteins
11

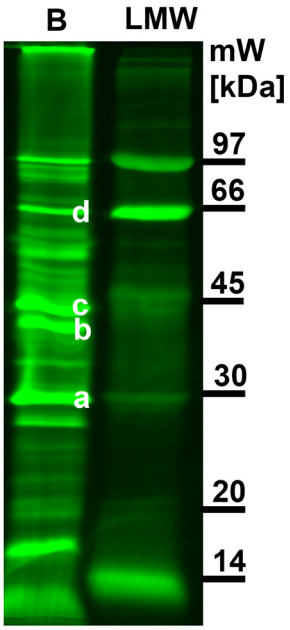
24A



24B



24C



24D

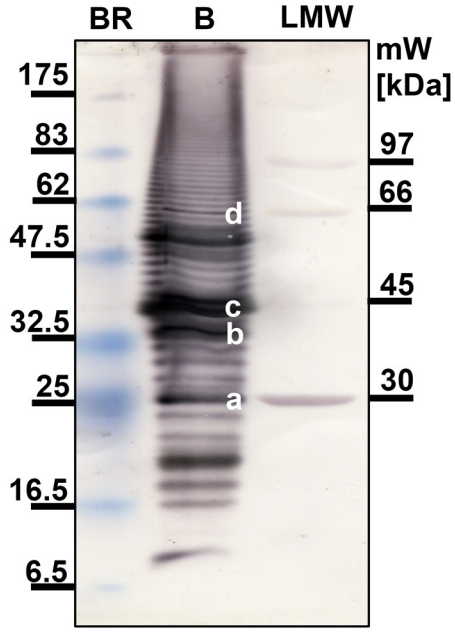


Fig. 21: Identification of immunogenic inner and outer membrane-associated proteins. Proteins obtained from *A. pleuropneumoniae* wt were labelled with Cy5 (red) and proteins from *A. pleuropneumoniae* Δ*arcA* were labelled with Cy3 (green). Equal amounts of *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* proteins were combined with the same amount of an internal standard (labelled with Cy 2 [blue], fluorescence not shown) comprising of inner and outer-membrane associated proteins from *A. pleuropneumoniae* wt, Δ*arcA* and Δ*hlyX* and were separated simultaneously by two dimensional gel electrophoresis. This was done for two independent biological repeats of *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* inner and outer membrane-associated proteins. Fluorescence was detected using a Typhoon™ Trio fluorescence scanner (21A, 21C). Both gels were blotted. The blot of gel 21A was incubated with a pool of sera from uninfected animals (21B). The blot of gel 21C was incubated with a pool of sera from pigs 21 days post experimental infection with *A. pleuropneumoniae* wt (21D). Immunogenic spots were assigned to the respective fluorescent spots and then identified from a preparative gel by mass spectrometry.

Fig. 22: Western blot identification of immunogenic outer membrane-associated proteins. 100 µg of *A. pleuropneumoniae* wt outer membrane-associated proteins were separated by two dimensional gel electrophoresis. The gels were blotted and one blotting membrane was incubated with a pool of sera from uninfected pigs (22A). The second membrane was incubated with a pool of sera obtained from animals 21 days post experimental infection (22B). The immunogenic protein spots were identified on a preparative gel, excised and identified by mass spectrometry.

Fig. 23: Identification of immunogenic secreted proteins. Secreted proteins obtained from *A. pleuropneumoniae* wt (Cy5 [red]) were combined with secreted proteins obtained from *A. pleuropneumoniae* Δ*hlyX* (Cy3 [green]) and an internal standard comprising secreted proteins from *A. pleuropneumoniae* wt, Δ*arcA* and Δ*hlyX* (Cy2 [blue], fluorescence not shown). These proteins were separated by two dimensional gel electrophoresis simultaneously. Fluorescence was detected using a Typhoon™ Trio fluorescence scanner (23A). Then the gel was blotted and the blotting membrane was first incubated with a pool of sera of uninfected pigs (23B) and second with a pool of sera obtained from pigs 21 days post infection (23C).

Fig. 24: Identification of immunogenic outer-membrane proteins. *A. pleuropneumoniae* outer membrane proteins were labelled with the green fluorescent dye Cy3. Equal amounts were separated in two lanes (lane A, B) of the same gel. Adjacent to each sample a low molecular weight marker (LMW) stained with the fluorescent dye Cy3 and a prestained broad range marker (BR) were loaded. Fluorescence was detected using a Typhoon™ Trio fluorescence scanner (24A, C). After Western blotting one membrane was incubated with a pool of preimmune sera (24B) and the other membrane was incubated with a pool of convalescent sera (24D). Same proteins of the LMW marker showed cross reaction with the convalescent sera. Immunogenic proteins were identified from a preparative gel by mass spectrometry. a: OmpA; b: OmpA and OmpP2; c: OmpA and OmpP2; d: FrpB.

Table 11: Immunogenic proteins of *A. pleuropneumoniae*.

Protein [a]	Protein [b]	MS # [c]	gel # [d]
inner and outer membrane-associated proteins (Fig. 21)			
RpsA	30S ribosomal protein S1	239	11
PflB	formate acetyltransferase	268	11
PflB	formate acetyltransferase	M40	11
PflB	formate acetyltransferase	M41	11
PflB	formate acetyltransferase	M42	11
LpdA	dihydrolipoyl dehydrogenase	M44	11
LpdA	dihydrolipoyl dehydrogenase	M45	11
DmsA	anaerobic dimethyl sulfoxide reductase chain A precursor	274	11
Adh2	aldehyde alcohol dehydrogenase 2	265	11
Adh2	aldehyde alcohol dehydrogenase 2	271	11
OmpA	outer membrane protein P5 precursor	256	11
OmpA	outer membrane protein P5 precursor	258	11
outer membrane-associated proteins (Fig. 22)			
ApxIIA	RTX-II toxin determinant A	M13	13
ApxIIA	RTX II toxin determinant A	M14; 230	13
ApxIIA	RTX II toxin determinant A	281	13
PckA	phosphoenolpyruvate carboxykinase ATP	M18	13
PckA	phosphoenolpyruvate carboxykinase ATP	M82	13
Pgm	phosphoglucomutase/phosphomannomutase	M93	13
Pgm	phosphoglucomutase/phosphomannomutase	M94	13
TufB	elongation factor Tu	M95	13
TufB	elongation factor Tu	M108	13
TufB	elongation factor Tu	M109	13
FkpA	putative FKBP-type peptidyl-prolyl cis-trans isomerase	M107	13
UshA	UshA precursor	M105	13
OmpP2	Outer membrane protein P2 precursor OMP P2	278	12
OmpP2	Outer membrane protein P2 precursor OMP P2	279	12
OmpA	outer membrane protein P5 precursor	282	13
OmpA	outer membrane protein P5 precursor	M29	13
Ape1960	hypothetical protein APL_1832	M110	13
secreted proteins (Fig. 23)			
ApxIIA	RTX-II toxin determinant A	M1	14
ApxIIA	RTX-II toxin determinant A	M2	14
ApxIIA	RTX-II toxin determinant A	M3	14
ApxIIA	RTX-II toxin determinant A	M4	14
ApxIIA	RTX-II toxin determinant A	M5	14
ApxIIA	RTX-II toxin determinant A	M6	14
ApxIIA	RTX-II toxin determinant A	M7	14
ApxIIA	RTX II toxin determinant A	154	14
FkpA	putative FKBP type peptidyl prolyl cis trans isomerase	123	14
OmpA	outer membrane protein P5 precursor	161	14
OmpA	outer membrane protein P5 precursor	151	14
OmpA	outer membrane protein P5 precursor OMP P5	153	14
outer membrane proteins (Fig. 24)			
OmpA	outer membrane protein P5 precursor and outer membrane protein P5 precursor OMP P5	79, 80	16
OmpA,	outer membrane protein P2 precursor OMP P2 and outer	73, 92,	15, 17

OmpP2	membrane protein P5 precursor and outer membrane protein P5 precursor OMP P5	93, 94	
OmpA, OmpP2	outer membrane protein P2 precursor OMP P2 and outer membrane protein P5 precursor and outer membrane protein P5 precursor OMP P5	81, 82, 83, 84, 85	16
FrpB	iron regulated outer membrane protein B	75	16

a) name of protein spot on gels and blots (Fig. 21 - 24)

b) result of mass spectrometry analysis.

c) number of the mass spectrometric analysis (Appendix G 6) that was performed for the identification of the protein of interest from the respective preparative gel [d]. Simple numbers indicate analysis by Q-TOF MSMS; numbers beginning with the capital letter M indicate analysis by MALDI-TOF MS.

d) This number indicates the preparative gel (Appendix G 5) from which the respective protein spot was obtained.

E Discussion

Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia causing high economic losses worldwide (Fenwick and Henry, 1994). Adaptation of the pathogen to its natural niche, the porcine respiratory tract and its pathogenicity are not completely understood. Recent studies showed that anaerobic metabolism generally is a virulence-associated trait in this respiratory tract pathogen (Baltes et al., 2003, Jacobsen et al., 2005, Baltes et al., 2005). Thus, the FNR homologue of *A. pleuropneumoniae*, HlyX, which is a global regulator for adaptation of gene expression to anaerobiosis, has been shown to be important for persistence of *A. pleuropneumoniae* in the infected lung (Baltes et al., 2005).

The aim of this study was to identify genes affected by oxygen-dependent transcription factors and to investigate how adaptation to anaerobiosis contributes to bacterial virulence. For this purpose, the global transcription factor for adaptation to anaerobic growth conditions, ArcA, was deleted in *A. pleuropneumoniae*. The *arcA* deletion mutant was characterized *in vivo* and *in vitro*. Subsequently, the HlyX and ArcA regulons were investigated by microarray analyses of anaerobically grown *A. pleuropneumoniae*, and these analyses were supported by a quantitative proteomic approach of different subcellular protein fractions based on 2D DIGE and quantitative 1D-PAGE in combination with mass spectrometry.

E 1 Characterization of *A. pleuropneumoniae* Δ*arcA* in vivo and in vitro

Since bacteria are able to sense low oxygen concentrations via the ArcAB system (Georgellis et al., 2001) and since ArcA-dependent regulation of virulence factors has been shown for the intestinal pathogen *Vibrio cholerae* (Sengupta et al., 2003), it was investigated whether a comparable relationship between virulence and ArcA also occurs in a respiratory tract pathogen.

The finding that the constructed *A. pleuropneumoniae* Δ*arcA* strain was highly attenuated initially led to the hypothesis that the physiological key features described for *arcA* mutants of *Enterobacteriaceae* (i.e. delayed growth under anaerobic [Sevcik et al., 2001] or under aerobic conditions [Oshima et al., 2002] or reduced long term survival [Nystrom et al., 1996]) might be responsible. However, contrary to its role in *Enterobacteriaceae* ArcA of *A. pleuropneumoniae* did neither influence growth nor survival and, therefore, appears to have different functions in *A. pleuropneumoniae*.

Deletion of ArcA reduced the ability of *A. pleuropneumoniae* for autoaggregation and biofilm formation under oxygen-deprived growth conditions, and both phenotypes are known

to be correlated (Schembri et al., 2003). Since biofilm formation has been shown to be common for field isolates of *A. pleuropneumoniae* (Kaplan and Mulks, 2005), it was hypothesized that the attenuation of *A. pleuropneumoniae* Δ*arcA* might be linked to this defect in autoaggregation and biofilm formation. To investigate possible causes for this defect, scanning electron microscopy was performed and a difference in threadlike extracellular material between *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*arcA* could be observed. This extracellular matrix - although present – appears to not be appropriately anchored to *A. pleuropneumoniae* Δ*arcA*. The matrix might be PGA, a linear exopolysaccharide composed of N-acetylglucosamine which, for *A. pleuropneumoniae*, has been described to be involved in biofilm formation by acting as an extracellular adhesin (Kaplan et al., 2004; Izano et al., 2007). Alternatively, the structures might be extracellular DNA as suggested to be present in *Haemophilus* (*H.*) *influenzae* (Jurcisek and Bakaletz, 2007) or adhesive pili as shown in *Actinobacillus actinomycetemcomitans* (Fine et al., 1999). A reason for the apparent defect in association of cells and matrix in *A. pleuropneumoniae* Δ*arcA* might be the lack of an ArcA regulated surface anchor or, alternatively, an upregulation of the biofilm releasing enzyme dispersin B (Kaplan et al., 2004) cutting the fibres of an extracellular N-acetylglucosamine polymer that binds to the bacterial surface. These results, which provide evidence for a direct influence of ArcA on biofilm formation, are supported by a recent report showing that the deletion of *arcA* in *E. coli* reduces competitiveness in biofilm formation (Junker et al., 2006).

Taken together, these results support the hypothesis that ArcA of *A. pleuropneumoniae* is less important in acute infection but plays an essential role in respiratory tract persistence of *A. pleuropneumoniae*. This implies that an in-depth investigation of the ArcA regulon in the respiratory tract pathogen *A. pleuropneumoniae* might lead to the identification of previously unknown persistence-associated factors and metabolic pathways and, therefore, improve our understanding of the mechanisms of host-pathogen interaction in chronic and latent infection.

E 2 The ArcA regulon of *A. pleuropneumoniae*

E 2.1 ArcA supports fumarate synthesis and use of fumarate as a terminal electron acceptor during anaerobiosis

Adaptation of gene expression to anaerobiosis controlled by the global transcriptional regulator ArcA has been shown to be important for virulence in different bacterial species (Boulette and Payne, 2007; Rickman et al., 2004; De Souza-Hart et al., 2003). In this study, an *arcA* deletion mutant of *A. pleuropneumoniae* was shown to be severely attenuated in

infection and defective in biofilm formation under oxygen-deprived growth conditions. In order to find clues for this phenotype, genome wide transcriptome and proteome analyses of anaerobically grown *A. pleuropneumoniae* wt in comparison to *A. pleuropneumoniae* Δ*arcA* were performed.

Based on the results obtained it was hypothesized that, under anaerobic conditions, i) *A. pleuropneumoniae* ArcA modulates the metabolism towards the synthesis of fumarate as terminal electron acceptor, and ii) glycerol-3-phosphate is used as reduction equivalent for reduction of fumarate and after oxidation to dihydroxy-acetone phosphate, serves as a precursor for fumarate synthesis (Fig. 25A).

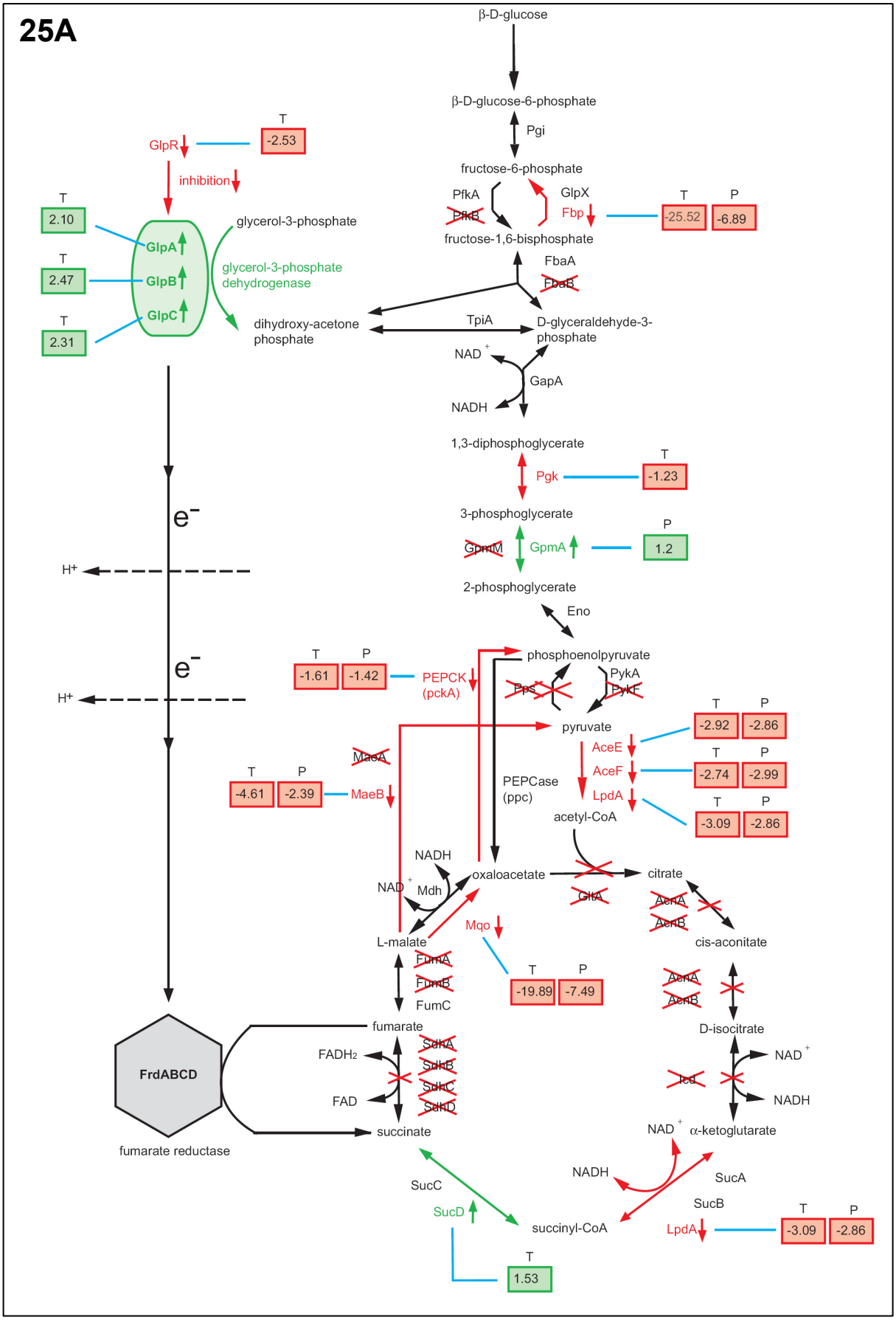
The glycolysis enzymes glycerol-3-phosphate dehydrogenase (GapA), phosphoglycerate kinase (Pgk), phosphoglyceromutase (GpmA) and pyruvate kinase (PykA) were found to be slightly increased whereas transcripts and proteins of the three subunits AceE, AceF and LpdA of the pyruvate dehydrogenase complex were identified to be downregulated about 3-fold by ArcA. The pyruvate dehydrogenase complex catalyzes the oxidative decarboxylation of pyruvate in order to generate acetyl-CoA for introduction of reduced carbon atoms into the citric acid cycle. However, a BLAST homology search using *E. coli* citric acid cycle enzymes revealed that *A. pleuropneumoniae* has a truncated citric acid cycle with no homologue for the citrate synthase (GltA) and, therefore, acetyl-CoA cannot be fused with oxaloacetate to form citrate. In addition, the enzymes catalyzing the transformation of citrate into α-ketoglutarate (AcnA, AcnB, Icd) are missing on the *A. pleuropneumoniae* genome. This inability to consume acetyl-CoA is hypothesized to lead to an accumulation of pyruvate and its precursor phosphoenolpyruvate. Pyruvate can be carboxylated by pyruvate carboxylase (Pyc) yielding oxaloacetate. Additionally, phosphoenolpyruvate can be introduced into the citric acid cycle intermediate oxaloacetate by phosphoenolpyruvate carboxylase (PepC)-mediated carboxylation. This requirement for CO_2 might be the cause why *A. pleuropneumoniae* grows best under microaerophilic conditions. The use of oxaloacetate for gluconeogenesis is unlikely since gluconeogenesis is strongly reduced by ArcA which is repressing two major steps. Thus, phosphoenolpyruvate carboxykinase (PckA) and fructose-1,6-bisphosphatase (Fbp) were found to be repressed by ArcA with the latter exhibiting a 25.5-fold repression of transcription and a 6.5-fold reduction of translation. Additionally, oxaloacetate cannot be consumed by the formation of citrate since *A. pleuropneumoniae* lacks a citrate synthase (GltA). Therefore, oxaloacetate is most likely converted to L-malate by malate dehydrogenase (Mdh), an enzyme favoring the NADH-consuming reduction of oxaloacetate to malate (Molenaar et al., 2000).

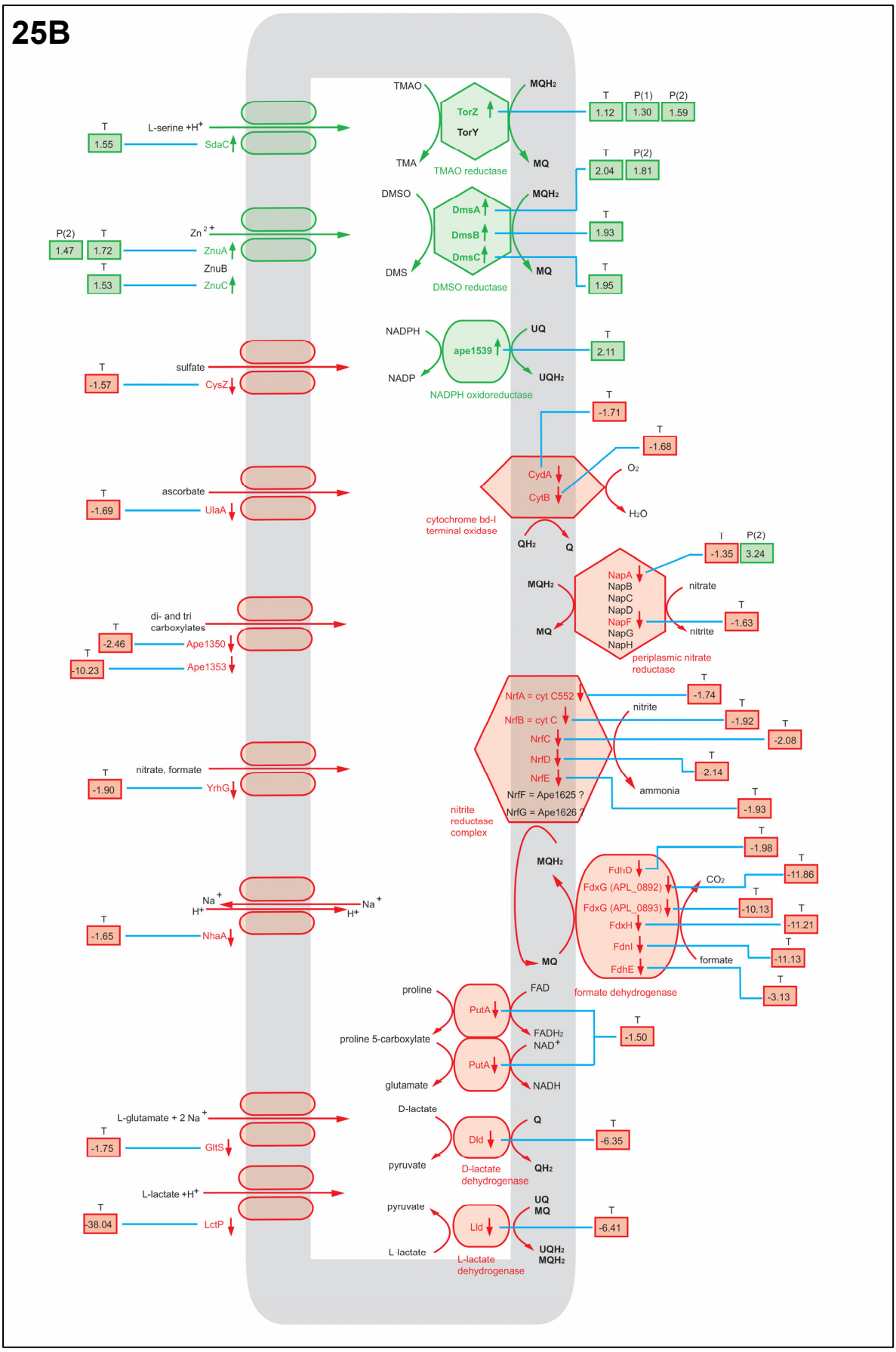
The membrane bound malate:quinone oxidoreductase (Mqo), an enzyme catalyzing the irreversible oxidation of malate (Kather et al., 2000), is severely downregulated (transcript: 20-fold; protein: 7.5-fold) in *A. pleuropneumoniae* by ArcA as it has also been observed for *E.*

coli (van der Rest et al., 2000). In addition, malic enzyme (MaeB) catalyzing the oxidative decarboxylation of malate into pyruvate was also downregulated (transcript: 4.6 fold; protein 2.4 to 3.3-fold) by ArcA, and the second decarboxylating malate dehydrogenase (MaeA) known from *E. coli* is missing in *A. pleuropneumoniae*. Therefore, L-malate is likely to be converted into fumarate by the FumC, the only fumarase found in *A. pleuropneumoniae*. Fumarate could then serve as the terminal electron acceptor in an electron transfer reaction of the respiratory chain catalyzed by the fumarate reductase enzyme complex (FrdABCD) (Boonstra et al., 1978). This hypothesis is supported by the fact that the FrdABCD complex is connected to the glycerol-3-phosphate-dehydrogenase through a simple electron transport chain (Schryvers and Weiner, 1981) transferring reduction equivalents from glycerol-3-phosphate to fumarate and thereby generating a proton gradient (Ingledew and Poole 1984) and that transcripts of the three proteins comprising the anaerobic glycerol-3-phosphate dehydrogenase, GlpABC, were found to be upregulated more then 2-fold by ArcA. This enzyme complex catalyzes the oxidation of glycerol-3-phosphate to dihydroxy-acetone phosphate which in turn is a glycolysis intermediate and can be converted into fumarate (Fig. 25A).

ArcA decreased the expression of dehydrogenases of the respiratory chain which require L-lactate (LldD), D-lactate (Dld), proline (PutA) or formate (FdhD, FdxG [APL_0892], FdxG [APL_0893], FdxH, FdnI, FdnE) as reduction equivalents. Additionally ArcA, downregulated the expression of terminal reductases using nitrite (NrfA, NrfB, NrfC, NrfD, NrfE), nitrate (NapA, NapF) or oxygen (CydA, CydB) as oxidation equivalents in the respiratory chain (Fig. 25B)

Under anaerobic conditions in the absence of terminal electron acceptors, fermentation is an alternative to respiration for maintaining continuous substrate chain phosphorylation during glycolysis. Thus, enzymes required for fermentation were identified as induced by anaerobiosis in *S. aureus* (Fuchs et al., 2007). ArcA of *A. pleuropneumoniae* instead reduced the ability for fermentation since the transcription of an alcohol dehydrogenase (AdhI) and an aldehyde dehydrogenase (AldA) were repressed during anaerobiosis by about 5 and 16-fold, respectively (Fig. 25C).


25A
β-D-glucose
β-D-glucose-6-phosphate
Pgi
fructose-6-phosphate
PfkA
PfkB
GlpX
Fbp
T
-25.52
P
-6.89
fructose-1,6-bisphosphate
FbaA
FbaB
GlpR
T
-2.53
inhibition
T
2.10
GlpA
T
2.47
GlpB
T
2.31
GlpC
glycerol-3-phosphate
glycerol-3-phosphate dehydrogenase
dihydroxy-acetone phosphate
TpiA
D-glyceraldehyde-3-phosphate
NAD+
GapA
NADH
1,3-diphosphoglycerate
Pgk
T
-1.23
3-phosphoglycerate
GpmM
GpmA
P
1.2
2-phosphoglycerate
Eno
e-
H+
e-
H+
T
-1.61
P
-1.42
PEPCK (pckA)
phosphoenolpyruvate
Pps
PykA
PykF
pyruvate
T
-2.92
P
-2.86
AceE
T
-2.74
P
-2.99
AceF
LpdA
T
-3.09
P
-2.86
acetyl-CoA
MaeA
T
-4.61
P
-2.39
MaeB
PEPCase (ppc)
NADH
NAD+
Mdh
oxaloacetate
GltA
citrate
AcnA
AcnB
cis-aconitate
Mqo
T
-19.89
P
-7.49
L-malate
FumA
FumB
FumC
fumarate
AcnA
AcnB
D-isocitrate
FADH2
SdhA
SdhB
SdhC
SdhD
FAD
succinate
Icd
NAD+
NADH
NAD+
α-ketoglutarate
FrdABCD
fumarate reductase
NADH
SucA
SucC
SucB
SucD
succinyl-CoA
LpdA
T
-3.09
P
-2.86
T
1.53


25B
TMAO
TorZ
TorY
TMA
TMAO reductase
MQH2
MQ
T 1.12 P(1) 1.30 P(2) 1.59
T 2.04 P(2) 1.81
DMSO
DmsA
DmsB
DmsC
DMS
DMSO reductase
T 1.93
T 1.95
NADPH
ape1539
NADP
NADPH oxidoreductase
UQ
UQH2
T 2.11
T -1.71
T -1.68
CydA
CytB
O2
H2O
cytochrome bd-I terminal oxidase
QH2
Q
T -1.35 P(2) 3.24
NapA
NapB
NapC
NapD
NapF
NapG
NapH
nitrate
nitrite
T -1.63
periplasmic nitrate reductase
NrfA = cyt C552
NrfB = cyt C
NrfC
NrfD
NrfE
NrfF = Ape1625 ?
NrfG = Ape1626 ?
nitrite reductase complex
T -1.74
T -1.92
T -2.08
T -2.14
ammonia
T -1.93
FdhD
FdxG (APL_0892)
FdxG (APL_0893)
FdxH
FdnI
FdhE
CO2
formate
formate dehydrogenase
T -1.98
T -11.86
T -10.13
T -11.21
T -11.13
T -3.13
proline
PutA
FAD
FADH2
NAD+
NADH
proline 5-carboxylate
glutamate
T -1.50
D-lactate
Dld
Q
QH2
pyruvate
D-lactate dehydrogenase
T -6.35
L-lactate
Lld
UQ
MQ
UQH2
MQH2
L-lactate dehydrogenase
T -6.41
T 1.55 L-serine +H+ SdaC
P(2) 1.47 T 1.72 Zn2+ ZnuA
T 1.53 ZnuB ZnuC
T -1.57 sulfate CysZ
T -1.69 ascorbate UlaA
T -2.46 di- and tri carboxylates Ape1350
T -10.23 Ape1353
T -1.90 nitrate, formate YrhG
Na+ H+ Na+ H+
T -1.65 NhaA
T -1.75 L-glutamate + 2 Na+ GltS
T -38.04 L-lactate +H+ LctP

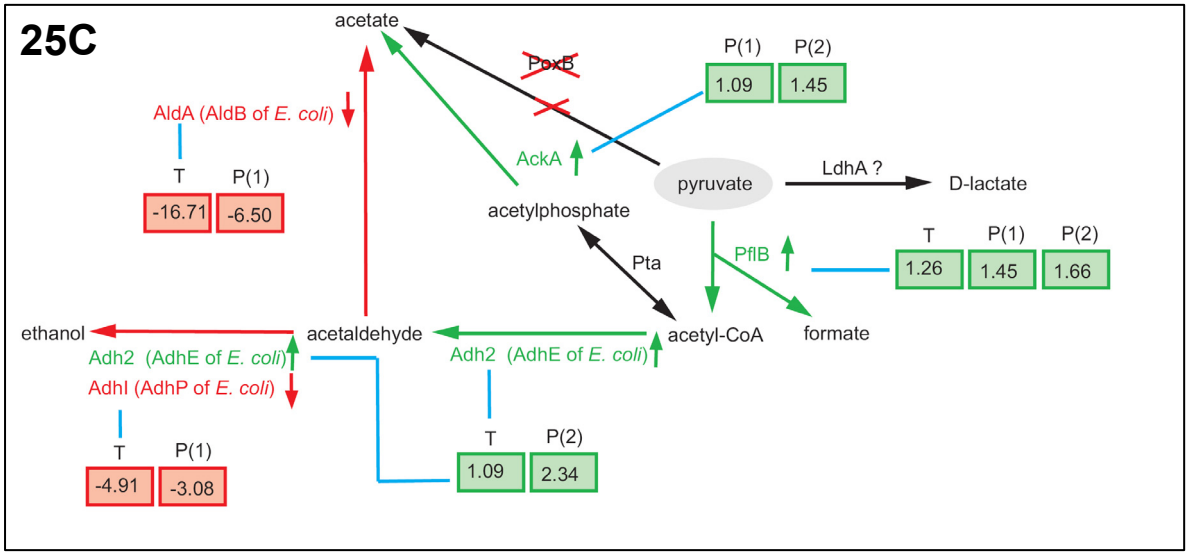


Fig. 25: Impact of ArcA regulated genes on metabolic pathways. A: Glycolysis and citric acid cycle; B: Dehydrogenases, terminal reductases and membrane transporter; C: Fermentation.

(A-C): T = transcript; P1 = protein preparations based on whole cell lysates; P2 = preparation of inner and outer membrane-associated proteins. The values within the boxes indicate the degree of ArcA dependent regulation (green box = upregulated by ArcA; red box = downregulated by ArcA) as obtained by microarray analysis (Transcript) or by 2D DIGE (Protein).

Dehydrogenases are indicated by a rounded rectangle, terminal reductases are indicated by hexagons and membrane transporters are indicated by a pair of rounded rectangles.

Biochemical pathways were adopted from *E. coli* K12 (Biocyc, http://www.biocyc.org). The genome of *A. pleuropneumoniae* serotype 5b strain L20 has recently been published (GenBank: CP000569) and annotation of the *A. pleuropneumoniae* genome is available at “Simple Yet Powerful Genome browser V 2.3” (http://informatics.bio.nrc.ca/ap5b). *E. coli* proteins were first searched using their annotation against the *A. pleuropneumoniae* annotated genome. When no homologues were found, the amino acid sequence of the *E. coli* protein was searched against the translated *A. pleuropneumoniae* genome. If *A. pleuropneumoniae* carried no homologue to the respective *E. coli* protein it is crossed out.

The pyruvate carboxylase of *A. pleuropneumoniae* is a homologue to the respective *Listeria monocytogenes* protein.

These cartoons were made using the Adobe® Illustrator® CS software.

Taken together, the ArcA regulon of *A. pleuropneumoniae* was identified. An ArcA deletion mutant of *A. pleuropneumoniae* was shown to be severely attenuated in infection and an analysis of the regulated genes implied that fumarate might be used as a terminal electron acceptor. While fumarate uptake is well known (Janausch et al., 2002), the synthesis of fumarate in order to use it for respiration as it is implied in this work for *A. pleuropneumoniae* has to my knowledge not been shown before. It has been reported that fumarate respiration is highly relevant for persistence and virulence of the enteric pathogen *Helicobacter* (*H*.) *pylori* in a mouse model (Ge et al., 2000). Therefore an ArcA regulated pathway promoting fumarate respiration under the reducing conditions in the diseased respiratory tract (Cantin et al., 1987; Day et al., 2004) is implied to be associated with virulence of *A. pleuropneumoniae*.

This hypothesis was proven in a follow-up project by Ibrahim Bendallah. He deleted the *frdA* gene encoding the fumarate reductase flavoprotein and tested the deletion mutant in an infection experiment of pigs. He could show that deletion of *frdA* significantly attenuated *A. pleuropneumoniae* in infection. His data strongly support the importance of fumarate respiration in virulence (unpublished data).

Higher eukaryotic organisms do not carry homologues to the bacterial fumarate reductase enzyme complex making this enzyme a suitable candidate for chemotherapy. A substance called nafuredin that is produced by *Aspergillus niger* (Ui et al., 2001) has been shown to be a potent inhibitor of the helminth fumarate reductase and showed antelminthic activity against *Haemonchus contortus* in *in vivo* trials with sheep (Omura et al., 2001). The finding that the fumarate reductase of *A. pleuropneumoniae* is important for virulence implies that nafuredin could have potential as a novel therapeutic drug to combat at least some bacterial infections.

E 2.2 Consideration of energy balance of glycerol-3-phosphate dependent fumarate reduction

The conversion of glucose (C6) into two molecules of fumarate (C4) requires the investment of two molecules CO_2. Energy is provided by one molecule phosphoenolpyruvate during phosphotransferase system (PTS)-mediated membrane transfer of glucose (Postma et al., 1993) and one molecule of ATP (6-phosphofructokinase). Two molecules ATP are generated by substrate chain phosphorylation during glycolysis (phosphoglycerate kinase) up to phosphoenolpyruvate. Phosphoenolpyruvate can then be carboxylated directly into oxaloacetate or by two steps via pyruvate. The latter pathway results in the synthesis of one ATP from the dephosphorylation of phosphoenolpyruvate to pyruvate and consumes one

ATP while carboxylating pyruvate into oxaloacetate. Taking the phosphoenolpyruvate which is invested during PTS-mediated phosphorylation of glucose as an equivalent for one ATP, then the net ATP balance is neutral. Per one molecule glucose entering glycolysis, two molecules of NADH are synthesized but later the formation of malate from oxaloacetate consumes these NADH. Thereby the pool of oxidized NAD^+ which is important for glycolysis is maintained.

The oxidation of glycerol-3-phosphate leads to the formation of dihydroxy-acetone phosphate, a glycolysis intermediate. If dihydroxy-acetone phosphate is used for fumarate synthesis a surplus of one molecule ATP is generated by substrate chain phosphorylation (no investment of phosphoenolpyruvate required).

The midpoint potentials for the glycerol-3-phosphate/dihydroxy-acetone phosphate and the fumarate/succinate redox pairs are -0.19 V and 0 V, respectively. The coupling of these two systems yields a $-\Delta G°$ of about 8.8 kcal (Miki and Lin 1975). This energy facilitates the export of approximately two protons from the cytosol into the periplasm thereby generating a proton gradient (Miki and Wilson 1978). Considering a stoichiometry of 3 to 4 protons transported per ATP synthesized (Fillingame 1990; van Walraven et al., 1996), the oxidation of two molecules of glycerol-3-phosphate coupled to the reduction of two molecules of fumarate leads to at least one molecule of ATP.

Taken together, one molecule glycerol-3-phosphate can produce one ATP by substrate chain phosphorylation and about 0.5 molecules of ATP by oxidative phosphorylation.

E 2.3 Comparison of the *A. pleuropneumoniae* ArcA regulon with ArcA regulons of other bacteria

The ArcA regulon of the close relative *H. influenzae* has been recently published (Wong et al., 2007). Only four genes were identified as being more than 2-fold upregulated by ArcA in *H. influenzae*. None of these positively regulated genes was found to be upregulated accordingly in *A. pleuropneumoniae* but instead 16 different genes were upregulated more than twofold. Nineteen genes were downregulated more then 2-fold in *H. influenzae* compared to 42 genes in *A. pleuropneumoniae* with the L-lactate permease gene (*lctP*) being the strongest negatively affected gene in both organism. Additionally, genes encoding L-lactate dehydrogenase (*lldD*) or formate dehydrogenase (*fdnI*, *fdxH*, *fdhE*) were found to be downregulated by ArcA in *H. influenzae* as well as in *A. pleuropneumoniae*.

Compared to global expression profiling experiments conducted with *E. coli* the number of ArcA regulated genes in *A. pleuropneumoniae* is relatively small. In *E. coli* transcription of 372 genes (Liu and De Wulf 2004) or 1139 genes (Salmon et al., 2005) were identified as

ArcA regulated under anaerobic growth conditions. The *E. coli* genome is about 2-fold larger as the *A. pleuropneumoniae* genome which, at least in part, accounts for the observed differences in size of the ArcA regulons. Additionally, compared to *E. coli A. pleuropneumoniae* inhabits a much more restricted environment (the porcine respiratory tract).

The comparison of ArcA regulated genes in *E. coli* and in *A. pleuropneumoniae* revealed that there are similarities and differences of the ArcA regulons possibly resulting in divergent strategies for anaerobic respiration.

ArcA of *E. coli* upregulated *cydAB*, encoding cytochrome d oxidase (Iuchi et al 1990a). This enzyme has a higher affinity for oxygen then cytochrome o oxidase and, therefore, is used under low oxygen concentrations (Anraku and Gennis 1987). Unlike *E. coli*, ArcA of *A. pleuropneumoniae* represses transcription of *cydAB* as it has also been observed for *Shewanella* (*S.*) *oneidensis* (Gralnick et al., 2005). The *dmsABC* genes encoding the DMSO reductase were identified as being upregulated by ArcA of *A. pleuropneumoniae* as it has been reported for the *dmsEFAB* of *S. oneidensis* (Gralnick et al., 2005). These genes, however, were not found to be ArcA-regulated in *E. coli*. The strongest ArcA-dependent upregulation in *A. pleuropneumoniae* was observed for a gene cluster encoding a putative methylation subunit type III restriction modification system. This regulation has not been observed in other organisms, and the function of this cluster is unknown to date.

E 3 The HlyX regulon of *A. pleuropneumoniae*

E 3.1 Adaptation of *A. pleuropneumoniae* metabolism to anaerobiosis by HlyX

Deletion of *hlyX* caused a severe attenuation of *A. pleuropneumoniae* in infection (Baltes et al., 2005). In order to identify the molecular mechanisms responsible for the attenuation of *A. pleuropneumoniae* Δ*hlyX*, the complete regulon was identified; *A. pleuropneumoniae* wt and *A. pleuropneumoniae* Δ*hlyX* were grown anaerobically, and gene expression was compared by microarray analyses. Additionally, a proteomic approach was performed to compare the protein patterns.

HlyX strongly increased the expression of several terminal reductases including DMSO reductase, TMAO reductase, nitrite reductase, and periplasmic nitrate reductase. With the exception of the latter, these reductases generate a proton gradient. In contrast, the periplasmic nitrate reductase acts as a terminal electron acceptor during anaerobiosis and, thereby, supports respiration of different carbon sources (Stewart and Darwin 2002). An *A. pleuropneumoniae* deletion mutant lacking the dimethyl sulfoxide reductase was reduced in

virulence (Baltes et al., 2003). Together, these finding indicate that *A. pleuropneumoniae* anaerobically adapts its metabolism by HlyX to use a variety of terminal electron acceptors for respiration of which at least some may be of importance during infection.

Analysis of the HlyX regulon revealed that four genes (*hybA*, *hybB*, *hyaA* and *hyaB*) encoding for a hydrogenase are strongly upregulated (more than 10-fold each). These genes exhibit strong homology to the *E. coli* hydrogenase 2 genes *hybA*, *hybB*, *hybO* and *hybC* (tested by BLAST analysis of the respective *E. coli* proteins against the *A. pleuropneumoniae* serotype 5b strain L20 complete annotated genome). Additionally, four genes necessary for hydrogenase maturation *(hypB*, *hypD*, *hypE* and *hypF* [Lutz et al., 1991; Reissmann et al., 2003]) are induced more than 2-fold by HlyX. The *A. pleuropneumoniae* genome carries no homologues to the formate hydrogenylase complex of *E. coli* that catalyzes the formation of hydrogen from formate during fermentative growth. Therefore, one option is that *A. pleuropneumoniae* uses exogenous hydrogen as a reduction equivalent for anaerobic growth. A possible source is hydrogen produced in the colon by bacterial fermentation of carbohydrates (Maier 2003) as part of this hydrogen is expelled by the lungs (Bond and Levitt 1972) and since it has been shown that this hydrogen supports virulence of *H. pylori* (Olson and Maier 2002). The H_2-splitting reaction catalyzed by hydrogenase contributes to the formation of a proton gradient across the membrane (Vignais et al., 2001). The electrons are transferred to different terminal electron acceptors (Laurinavichene and Tsygankov 2001). A hydrogenase- and fumarate reductase-coupled reaction has been identified to transfer electrons from hydrogen to fumarate and thereby generating a proton motive force in *E. coli* (Macy et al., 1976). However, electrons originating from hydrogen appear to not to play a major role for anaerobic respiration during infection with *A. pleuropneumoniae* since the deletion of *hybB* caused no reduction in virulence (Baltes et al., 2004b).

In addition to its impact on anaerobic metabolism, HlyX of *A. pleuropneumoniae* is a strong regulator of iron acquisition genes. Iron acquisition in *Neisseriaceae* and *Pasteurellaceae* is mediated by a complicated system allowing utilization of transferrin-bound ferric (Fe^{3+}) iron. Transferrin binds to bacterial outer membrane receptors (TbpA, TbpB). The iron atom is then transferred into the cytosol by an energy dependent process needing TonB and an inner membrane protein complex like ExbBD (Perkins-Balding, et al., 2004). In this study the entire system has been identified to be strongly positively regulated by HlyX. The reason for the HlyX dependent anaerobic induction of this iron uptake system is unclear. The infection with *A. pleuropneumoniae* causes the formation of encapsulated sequesters in the porcine lung. These pathologic areas are poorly supplied with blood and therefore with serum soluble transferrin. It seems possible that during evolution of *A. pleuropneumoniae* the transferrin-bound iron uptake system got under control of oxygen dependent regulators since the decline in oxygen availability is accompanied *in vivo* by a decrease in iron supply.

E 3.2 Comparison of the *A. pleuropneumoniae* HlyX regulon with FNR regulons of other bacteria

FNR of *E. coli* affects the expression of 502 genes by more then 2-fold, as shown for different growth conditions (Constantinidou et al., 2006). The FNR regulon of the closer relative to *A. pleuropneumoniae*, *Neisseria* (*N.*) *gonorrhoeae* is much smaller and contains only 14 genes that are activated as well as six genes that are repressed by FNR (Whitehead et al., 2007). In contrast, the HlyX regulon of *A. pleuropneumoniae* comprising 418 genes that are regulated more then 2-fold is large. Although *A. pleuropneumoniae* Δ*hlyX* as well as the parent strain were harvested in their logarithmic growth phase it can not be excluded that at least part of these genes showed an expression difference due to the reduced growth of the mutant strain. The gene *ape0761* encoding for a putative methylation subunit of a type III restriction modification system is the strongest positively regulated gene in the HlyX operon (29.8-fold) as well as in the ArcA operon (16.5-fold) of *A. pleuropneumoniae*. A homologue to this gene was also identified as positively regulated by HlyX of *N. gonorrhoeae* but to a much lower extend (2.9-fold) (Whitehead et al., 2007). Terminal reductases were identified to be positively regulated by FNR in different bacterial species. Thus, the nitrite reductase is also upregulated by HlyX of *N. gonorrhoeae* (Whitehead et al., 2007). DMSO reductase encoding genes were also found to be strongly induced by FNR in *Salmonella* (*S.*) *enterica* (Fink, et al., 2007), and, as in *A. pleuropneumoniae*, FNR is a positive regulator for the expression of virulence associated hydrogenases in *S. enterica* (Zbel et al., 2007).

Genes encoding for the glycerol-3-phosphate dehydrogenase and fumarate reductase enzyme complex that built a short respiratory chain on its own have been identified to be positively regulated by FNR in *E. coli* (Jones and Gunsalus 1987; Iuchi et al., 1990b). However, this dehydrogenase / terminal reductase pair is downregulated by HlyX of *A. pleuropneumoniae* and it remains elusive why neither ArcA nor HlyX are able to activate frdABCD transcription. One reason could be that transcription from some FNR dependent promoters can be inhibited by catabolite repression in the presence of glucose as shown for the *nrf* promoter of *E. coli* (Browning et al., 2005). Since *A. pleuropneumoniae* has been grown in a rich medium catabolite repression might account for repression of some HlyX regulated genes.

E 4 Comparison of ArcA- and HlyX-mediated adaptation of gene expression

The ArcAB two-component system and FNR (HlyX in *A. pleuropneumoniae*) are major transcription factors required for bacterial adaptation to anaerobiosis. The regulons of both

transcriptions factors have been identified in this study by transcriptomic and proteomic analyses. Expression of 903 genes is significantly affected by HlyX more than 1.5-fold, and expression of 199 genes is significantly affected by ArcA more than 1.5-fold. Both regulon share 114 genes affected by both transcription factors. Consequently, ArcA affects 12.6 % of the HlyX regulon, and HlyX affects 57 % of the ArcA regulon. The expression of 103 genes is coregulated by ArcA and HlyX (64 positive, 39 negative). However, 11 genes are differentially affected by both regulatory proteins. Two subunits of the glycerol-3-phosphate dehydrogenase enzymes complex (*glpA*, *glpB*) are upregulated by ArcA but downregulated by HlyX. The glycerol-3-phosphate dehydrogenase has been considered as being linked to the fumarate reductase enzyme complex thereby providing the reduction equivalents required for anaerobic use of fumarate as a terminal electron acceptor. The fumarate reductase (*frdA*, *frdB*, *frdC*) was also identified to be slightly (below 2-fold) downregulated by HlyX. This implies that HlyX, in contrast to ArcA, favors the use of terminal electron acceptors different to fumarate under anaerobic growth conditions. This hypothesis is supported by the following observations: i) the three genes *dmsA*, *dmsB*, *dmsC*, encoding DMSO reductase are strongly upregulated by HlyX but only slightly by ArcA. ii) the genes *nrfA*, *nrfB* and *nrfD* encoding a nitrite reductase are downregulated by ArcA but are up to 20-fold upregulated by HlyX. iii) the gene *napA* encoding the large subunit of the periplasmic nitrate reductase and the *napF* gene encoding a stimulator for the periplasmic nitrate reductase are downregulated by ArcA (1.35-fold and 1.63-fold, respectively) but these genes are strongly upregulated by HlyX (14.9-fold and 6.9-fold, respectively). iv) the TMAO reductase encoding gene *torZ* is only 1.12-fold upregulated by ArcA but 11-fold by HlyX. Expression of the gene *torY* encoding the second subunit of TMAO reductase is upregulated 17.6-fold by HlyX (Fig. 26).

Considering the degree of up- and downregulation, ArcA is a predominantly negative regulator, whereas HlyX mainly acts as a positive regulator in *A. pleuropneumoniae* under anaerobic conditions. HlyX increased the expression of 30 genes by a factor above 7 or more, compared to a single gene upregulated to that extend by ArcA. In contrast, no gene is repressed by HlyX more than 7 fold compared to 13 genes that are repressed by ArcA more than 7-fold despite the fact that the ArcA regulon contains only 22 % of the number of genes of the HlyX regulon (Fig. 27).

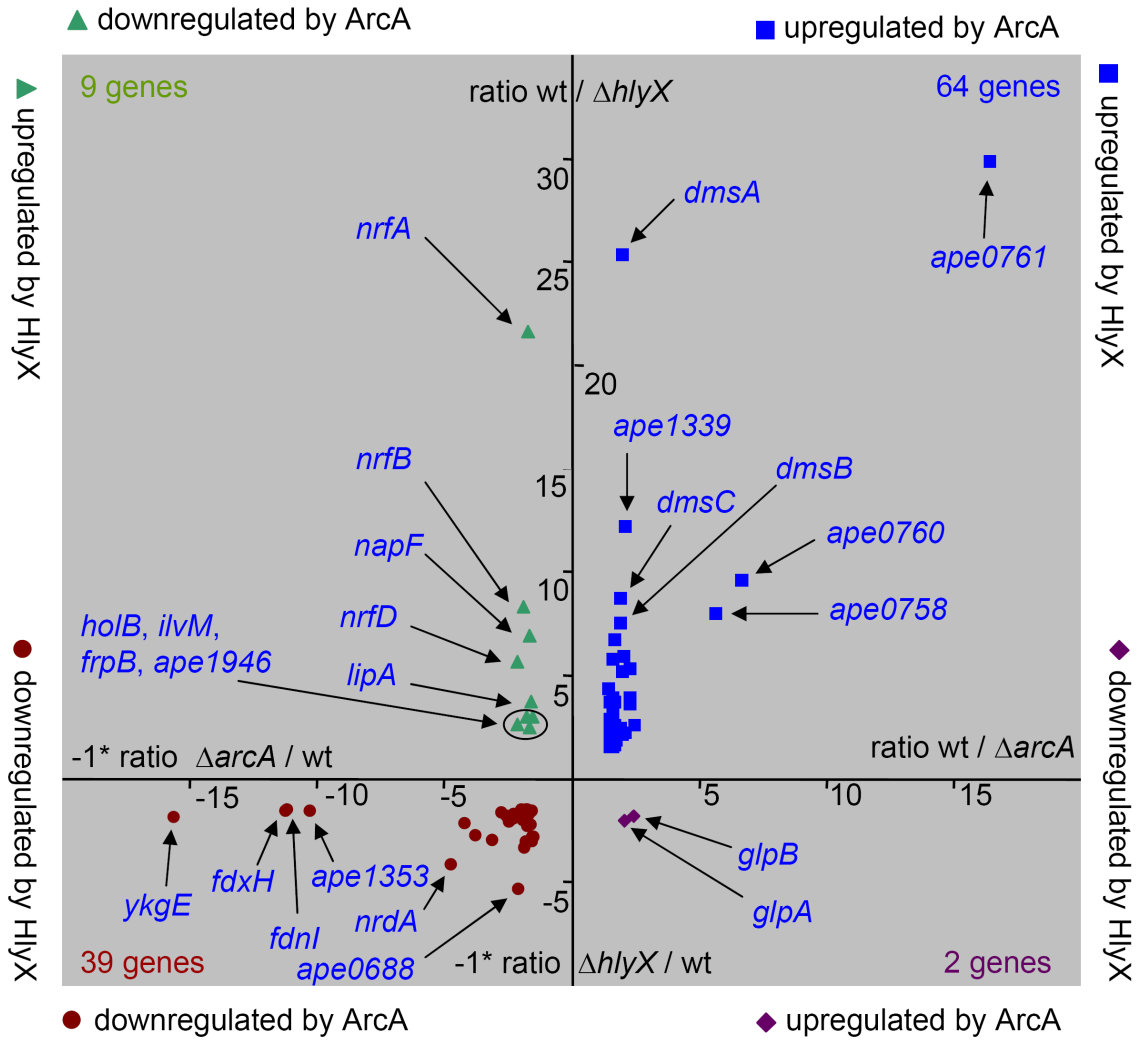


Fig. 26: Comparison of gene regulation by ArcA and HlyX. Transcripts that were found to be upregulated by ArcA and upregulated by HlyX got positive values and are shown in the upper right quarter of this graph. Transcripts that were downregulated by both regulators got negative values and are shown in the lower left quarter. Genes that were not unanimously regulated by both transcription factors appear either in the upper left (upregulated by HlyX and downregulated by ArcA) or lower right (upregulated by ArcA and downregulated by HlyX) quarter of this graph. On the x- and y-axis the ratios of transcripts between *A. pleuropneumoniae* wt and the respective mutant are shown. This ratio was calculated as wt / mutant for genes upregulated by the respective transcription factor or as -1 x mutant / wt for genes downregulated by the respective transcription factor. Genes whose regulation between ArcA and HlyX deviated strongly are highlighted by arrows.

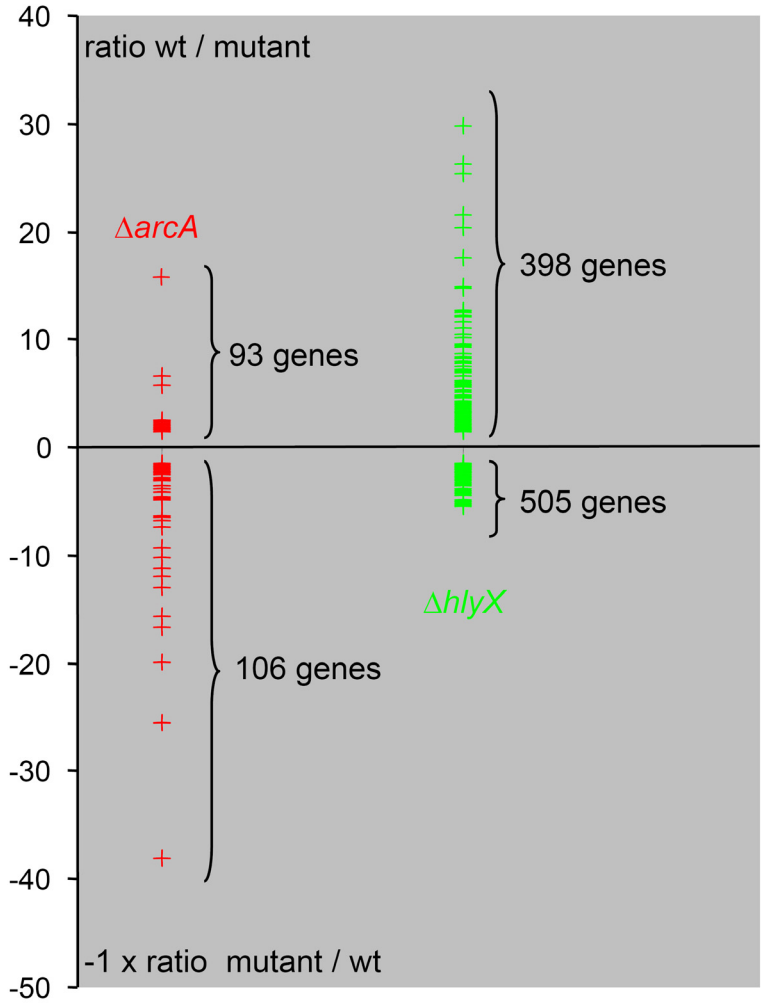


Fig. 27: Comparison of the degree of gene regulation by ArcA and HlyX. The ratio between wt and mutant is shown on the x-axis. Genes upregulated by ArcA or HlyX got positive values, those downregulated got negative values.

HlyX and ArcA are activated due to a decline in oxygen availability. It has been reported that in *E. coli*, ArcA exerts its control in a range from 10 to 20% oxygen, whereas FNR is active at 0 to 10 % oxygen (Tseng et al., 1996). However, another study showed that activity of ArcA and FNR increased in a comparable manner as a result of a decline in oxygen tension (Becker et al., 1996).

ArcA regulates the expression of 110 genes under aerobic conditions in *E. coli* and can be activated by interaction with other two-component systems (Oshima et al., 2002). It remains unclear whether ArcA and HlyX are active simultaneously in the natural niche of the porcine respiratory tract and complement each other. However, a combinatory effect of oxygen and various other factors might contribute to selective activation of a certain regulator facilitating the best adaptation of *A. pleuropneumoniae* to its host.

Identification of ArcA and HlyX regulated genes was conducted by comparing gene and protein expression between the wt strain and the respective mutant upon growth under anaerobic conditions in a rich medium. This approach is not sufficient to determine whether the genes identified are under direct transcriptional control of the respective regulator or whether the alteration of expression is an indirect effect caused by deletion of the regulatory protein. Additionally, it is likely that different environmental conditions may induce different subsets of ArcA or HlyX-regulated genes due to interaction with other regulatory systems. However, for the conditions tested, the influence of ArcA and HlyX on gene expression was clearly identified permitting conclusions for ArcA and HlyX regulation of gene expression under conditions present in the natural environment of the porcine respiratory tract.

E 5 Shed membrane vesicles (blebs) appear to be caught in the biofilm matrix of *A. pleuropneumoniae*

A. pleuropneumoniae wt, in contrast to *A. pleuropneumoniae* ∆*arcA*, has been shown in this study to form autoaggregates under anaerobic conditions and to form biofilm. Additionally, it was shown that *A. pleuropneumoniae* ∆*hlyX* grew significantly slower then the wt strain or the *arcA* deletion mutant and showed no autoaggregation at 6 h post inoculation of an anaerobic liquid culture which was used for protein preparation; however, autoaggregation was clearly visible 10 h post inoculation. The 2D DIGE analysis of different protein preparations of the three strains generally supported the results obtained by the microarray approach. However, for some proteins a considerable enrichment was observed solely in the detergent-based protein preparations of *A. pleuropneumoniae* wt compared to *A. pleuropneumoniae* ∆*arcA* and *A. pleuropneumoniae* ∆*hlyX* and led to the hypothesis that this might be due to the autoaggregative growth of the wt strain. Thus, all proteins enriched

by detergent from *A. pleuropneumoniae* wt are predicted to be either located in the periplasm, or outer membrane-associated or secreted, including the periplasmic nitrate reductase (NapA), the outer membrane protein P2 (OmpP2) and the outer membrane proteins P5 and OMP P5 (both were designated as OmpA), the iron regulated outer membrane protein B (FrpB), and the ApxIIA toxin. The protein OmpA was identified to be a biofilm component of *H. influenzae* (Gallaher et al., 2006), and OmpA as well as OmpP2 were found to be expressed in membranes of *H. influenzae* grown as a biofilm (Murphy and Kirkham 2002). OmpA is a regulator of biofilm formation in *E. coli* (Barrios et al., 2005). The possible reason for an enrichment of these proteins in biofilm could be the formation of membrane vesicles (blebs). The proteins FrpB, OmpP2 and OmpA are cationic outer membrane associated proteins (pI > 8.9), and NapA is a basic periplasmic membrane associated protein (pI = 8.2). In addition, a characterization of shed membrane vesicles ("blebs") of *N. meningitidis* revealed that nearly 75 % of their protein content are outer membrane proteins and that the majority of bleb proteins are highly basic. A homologue of FrpB of *A. pleuropneumoniae* was identified as a major bleb component of *N. meningitidis* (Post et al., 2005). The formation of blebs is an envelope stress response occurring in the host (McBroom and Kuehn 2007), and blebs have been described as delivery vehicles for toxins (Kuehn and Kesty 2005) including ApxI of *A. pleuropneumoniae* (Negrete-Abascal et al., 2000) and quorum sensing signals (Mashburn and Whiteley 2005). It is hypothesized that membrane vesicles shed by *A. pleuropneumoniae* wt are, at least in part, caught in the extracellular matrix of clumping bacteria, whereas blebs formed by *A. pleuropneumoniae* Δ*arcA* and *A. pleuropneumoniae* Δ*hlyX* are not retained, since these deletion mutants do not autoaggregate. This local enrichment of Apx toxin containing blebs in the biofilm matrix could explain the severe local tissue destruction observed during *A. pleuropneumoniae* infection. This tissue destruction supports the release of nutrients and iron from lysed cells and it appears not unlikely that bleb associated FrpB contributes to supply iron to *A. pleuropneumoniae* (Beucher et al., 1995).

Taken together these data imply that one function of biofilm in virulence is the trapping of outer membrane vesicles enriched with Apx toxins, the iron scavenging protein FrpB (Beucher and Sparling 1995) and possibly other virulence associated factors.

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G Appendix

G 1 Genes upregulated by ArcA as obtained by microarray analysis

gene	locus tag	gene product	COG #	COG	EC #	psorb_loc	location	T-test	ratio
ape0761	APL_0705	putative methylation subunit, type III restriction-modification system	COG2189	L	2.1.1.72	OuterMembrane	813144..816032 [-]	6.39E-07	**16.46**
ape0760	APL_0704	hypothetical protein					809428..812742 [-]	1.03E-08	**6.72**
ape0758	APL_0703	hypothetical ATP-dependent helicase	COG0553	KL		Cytoplasmic	806555..809425 [-]	1.12E-03	**5.71**
serC	APL_0702	phosphoserine aminotransferase	COG1932	HE	2.6.1.52	Cytoplasmic	805273..806361 [-]	5.08E-08	**2.51**
glpB	APL_0380	anaerobic glycerol-3-phosphate dehydrogenase subunit B	COG3075	E	1.1.99.		432880..434166 [+]	3.42E-03	**2.47**
htpG	APL_0987	chaperone protein HtpG	COG0326	O		Cytoplasmic	1139729..1141606 [-]	9.50E-06	**2.34**
dnaK	APL_1906	chaperone protein dnaK	COG0443	O		Cytoplasmic	2136557..2138455 [-]	4.67E-05	**2.32**
glpC	APL_0381	anaerobic glycerol-3-phosphate dehydrogenase subunit C	COG0247	C	1.1.99.5		434163..435437 [+]	8.57E-04	**2.31**
ubiG	APL_0285	3-demethylubiquinone-9,3-methyltransferase	COG2227	H	2.1.1.64		314720..315424 [-]	5.33E-10	**2.30**
ape0999	APL_0920	hypothetical protein					1061681..1062937 [+]	1.66E-05	**2.15**
ape1539	APL_1432	putative NAD(P)H oxidoreductase	COG2249	R	1.6.99.-		1637523..1638098 [+]	7.92E-05	**2.11**
glpA	APL_0379	anaerobic glycerol-3-phosphate dehydrogenase subunit A	COG0578	C	1.1.99.5	Cytoplasmic	431205..432890 [+]	1.92E-03	**2.10**
ape2007	APL_1878	hypothetical protein				OuterMembrane	2106838..2107857 [+]	4.88E-04	**2.07**
dmsA	APL_1674	anaerobc dimethyl sulfoxide reductase chain A precursor	COG0243	C	1.8.99.-		1900092..1902509 [+]	2.28E-05	**2.04**
ape1501	APL_1396	hypothetical protein					1596821..1597336 [+]	3.00E-05	**2.02**
ape2008	APL_1879	hypothetical protein	COG1629				2107994..2109331 [+]	1.40E-04	**2.00**
potD2	APL_0368	spermidine/putrescine-binding periplasmic protein 1 precursor	COG0687	E		Periplasmic	413401..414483 [+]	6.85E-07	**1.98**
infA	APL_1228	translation initiation factor IF-1	COG0361	J			1409885..1410103 [-]	2.80E-04	**1.98**
gntR	APL_0571	HTH-type transcriptional regulator	COG1167	KE		Cytoplasmic	643793..645193 [-]	2.59E-05	**1.96**
dmsC	APL_1676	anaerobic dimethyl sulfoxide reductase chain C	COG3302	R		CytoplasmicMembrane	1903138..1903971 [+]	1.58E-07	**1.95**
dmsB	APL_1675	anaerobic dimethyl sulfoxide reductase chain B	COG0437	C		CytoplasmicMembrane	1902519..1903136 [+]	7.48E-07	**1.93**
ape0505	APL_0471	hypothetical protein					542535..542828 [-]	7.43E-03	**1.79**
afuA_2	APL_0563	Fe3+ ABC transporter, iron-binding protein	COG1840	P		Periplasmic	631946..632977 [+]	9.47E-06	**1.76**
ape1572	APL_1463	putative ABC transporter					1676054..1676845 [+]	1.00E-02	**1.76**
smpA	APL_0428	small protein A	COG2913	J			485468..485812 [-]	7.16E-05	**1.74**
znuA	APL_1440	high-affinity zinc uptake system protein ZnuA precursor	COG4531	P		Periplasmic	1647160..1648137 [+]	5.94E-06	**1.72**
accD	APL_0631	acetyl-coenzyme A carboxylase carboxyl transferase subunit beta	COG0777	I	6.4.1.2		718567..719460 [+]	2.12E-05	**1.72**
groEL	APL_1012	60 kDa chaperonin	COG0459	O		Cytoplasmic	1178033..1179676 [-]	2.82E-05	**1.72**
sspB	APL_0657	stringent starvation protein B	COG2969	R			751608..752024 [-]	2.84E-12	**1.71**
era	APL_0544	GTP-binding protein Era-like	COG1159	R		Cytoplasmic	614750..615664 [+]	1.06E-07	**1.70**
lpxA	APL_0407	Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase	COG1043	M	2.3.1.129	Cytoplasmic	464325..465119 [-]	8.48E-05	**1.70**
glnA	APL_1941	Glutamine synthetase	COG0174	E	6.3.1.2	Cytoplasmic	2172693..2174108 [-]	4.68E-11	**1.69**
ape0031	APL_0029	ABC transporter periplasmic protein	COG0747	E		Periplasmic	33833..35407 [-]	1.01E-03	**1.69**
ape0215	APL_0205	predicted rRNA methyltransferase	COG0566	J	2.1.1.-		223489..224526 [-]	1.00E-07	**1.69**
dcd	APL_0835	deoxycytidine triphosphate deaminase	COG0717	F	3.5.4.13	Cytoplasmic	968300..968884 [+]	1.57E-06	**1.68**
fis	APL_0190	DNA-binding protein Fis	COG2901	KL			210047..210343 [+]	4.00E-05	**1.66**
dnaJ	APL_1905	Chaperone protein dnaJ	COG0484	O		Cytoplasmic	2135185..2136327 [-]	2.32E-05	**1.66**
ape1150	APL_1061	hypothetical protein					1230590..1231186 [+]	2.86E-03	**1.66**
ape1059	APL_0975	hypothetical protein	COG0037	D		Cytoplasmic	1128272..1129228 [+]	3.66E-05	**1.65**
argD	APL_1974	Diaminobutyrate--2-oxoglutarate aminotransferase	COG0160	E	2.6.1.76		2200641..2201936 [+]	5.71E-09	**1.65**
ape1794	APL_1677	hypothetical protein	COG3381	R			1904027..1904632 [+]	1.56E-06	**1.65**
recA	APL_1143	recombinase A	COG0468	L		Cytoplasmic	1318766..1319896 [-]	8.83E-05	**1.65**
pdxT	APL_0573	glutamine amidotransferase subunit PdxT	COG0311	H	2.6.-.-		646167..646742 [+]	5.77E-05	**1.64**
ape0978	APL_0905	hypothetical protein					1047346..1047684 [-]	2.27E-03	**1.63**

ilvH	APL_0728	acetolactate synthase small subunit	COG0440	E	2.2.1.6	Cytoplasmic	836810..837301 [+]	3.89E-05	**1.63**
ape2168	APL_2031	hypothetical protein	COG0526			Cytoplasmic	2257942..2258436 [-]	3.93E-03	**1.63**
pdxS	APL_0572	pyridoxal biosynthesis lyase PdxS	COG0214	H	4.-.-.-	Cytoplasmic	645280..646167 [+]	2.71E-05	**1.63**
ape1328	APL_1231	hypothetical protein				Cytoplasmic	1411876..1412367 [+]	4.24E-04	**1.62**
ape0311	APL_0295	hypothetical protein	COG1179	H			327747..328511 [+]	3.16E-04	**1.62**
recO	APL_0545	DNA repair protein RecO	COG1381	L			615674..616396 [+]	6.56E-08	**1.62**
ape0309	APL_0293	putative type I site-specific restriction-modification system, R (restriction) subunit	COG0610	V			322648..325785 [+]	2.63E-06	**1.62**
ape0487	APL_0453	hypothetical protein					521551..522417 [-]	4.21E-06	**1.61**
rnc	APL_0543	ribonuclease III	COG0571	K	3.1.26.3	Cytoplasmic	614009..614680 [+]	5.69E-09	**1.61**
narQ	APL_0462	sensor protein NarQ-like	COG3850	T	2.7.3.-	CytoplasmicMembrane	533862..535577 [+]	6.62E-05	**1.61**
cpxR	APL_0629	transcriptional regulatory protein CpxR	COG0745	TK		Cytoplasmic	716776..717504 [-]	7.52E-04	**1.60**
ape1308	APL_1211	hypothetical protein					1393801..1394637 [+]	1.08E-04	**1.59**
recJ	APL_0459	single-stranded-DNA-specific exonuclease RecJ	COG0608	L	3.1.-.-	Cytoplasmic	530220..531977 [+]	1.93E-04	**1.59**
cysE	APL_1511	serine acetyltransferase	COG1045	E	2.3.1.30	Cytoplasmic	1726968..1727783 [+]	3.19E-06	**1.59**
trxA	APL_1078	thioredoxin	COG0526	OC		Cytoplasmic	1247188..1247505 [-]	1.62E-04	**1.59**
pheA	APL_1033	P-protein [Includes: Chorismate mutase; Prephenate dehydratase]	COG0077	E	4.2.1.51 and 5.4.99.5	Cytoplasmic	1198612..1199769 [-]	9.31E-04	**1.59**
ape0247	APL_0236	putative lipoprotein	COG3015	MP			254045..254419 [-]	1.11E-03	**1.59**
ape0752	APL_0697	putative HTH-type transcriptional regulator	COG0583	K		Cytoplasmic	798742..799638 [+]	9.90E-05	**1.58**
lon	APL_0376	ATP-dependent protease La	COG0466	O	3.4.21.53	Cytoplasmic	425663..428071 [-]	2.27E-03	**1.58**
fabZ	APL_0408	(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase	COG0764	I	4.2.1.-	Cytoplasmic	465147..465611 [-]	4.74E-06	**1.58**
fabA	APL_1889	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase	COG0764	I	4.2.1.60	Cytoplasmic	2121238..2121768 [-]	6.27E-04	**1.57**
clpB	APL_1087	chaperone ClpB	COG0542	O		Cytoplasmic	1257302..1259875 [+]	1.02E-02	**1.55**
ape1017	APL_0937	hypothetical protein				Cytoplasmic	1079048..1079590 [-]	2.66E-04	**1.55**
sdaC	APL_0856	serine transporter	COG0814	E		CytoplasmicMembrane	995022..996251 [+]	3.21E-04	**1.55**
uupA	APL_0294	ABC transporter ATP-binding protein Uup-1	COG0488	R		CytoplasmicMembrane	325804..327747 [+]	1.38E-04	**1.55**
arcA	APL_0048	aerobic respiration control protein ArcA	COG0745	TK		Cytoplasmic	54844..55560 [+]	5.60E-06	**1.54**
asnC	APL_1838	Regulatory protein asnC	COG1522	K		Cytoplasmic	2062188..2062649 [+]	2.47E-07	**1.54**
mukB	APL_0581	chromosome partition protein MukB	COG3098	D		OuterMembrane	657485..661975 [+]	3.35E-08	**1.54**
ape0960	APL_0889	hypothetical protein					1029866..1031281 [+]	7.93E-05	**1.54**
sucD	APL_0451	succinyl-CoA ligase [ADP-forming] subunit alpha	COG0074	C	6.2.1.5	Cytoplasmic	519366..520238 [-]	5.82E-09	**1.53**
znuC	APL_0456	high-affinity zinc uptake system ATP-binding protein ZnuC	COG1121	P		Cytoplasmic	526962..527762 [-]	3.10E-04	**1.53**
ape0072	APL_0070	hypothetical protein					83031..83663 [-]	1.42E-04	**1.53**
ape0841	APL_0781	putative ATP-dependent helicase	COG1199	KL	3.6.1.-		897087..899009 [-]	2.64E-04	**1.53**
ppiB	APL_0914	peptidyl-prolyl cis-trans isomerase B	COG0652	O	5.2.1.8	Cytoplasmic	1055847..1056356 [+]	2.47E-04	**1.52**
ape0293	APL_0278	putative Mg-dependent deoxyribonuclease	COG0084	L	3.1.21.-	Cytoplasmic	306917..307669 [+]	9.13E-05	**1.52**
ape0840	APL_0780	hypothetical protein	COG1380	R		CytoplasmicMembrane	896661..897020 [-]	2.90E-03	**1.52**
mtfA	APL_0685	putative RNA 2'-O-ribose methyltransferase MtfA	COG2933	R		Cytoplasmic	783146..784237 [-]	9.88E-11	**1.52**
ape0497	APL_0463	predicted sortase and related acyltransferases	COG1247	M	2.3.1.-		535600..536118 [+]	1.11E-03	**1.52**
ape0283	APL_0270	hypothetical protein					298665..299156 [+]	1.05E-04	**1.52**
holA	APL_0874	DNA polymerase III subunit delta			2.7.7.7		1015440..1016462 [+]	5.43E-08	**1.52**
ape1373	APL_1273	putative fimbrial biogenesis and twitching motility protein PilF-like protein	COG3063	NU			1464050..1464598 [-]	1.94E-06	**1.51**
groES	APL_1013	10 kDa chaperonin	COG0234	O		Cytoplasmic	1179740..1180030 [-]	5.09E-03	**1.51**
serB	APL_1230	phosphoserine phosphatase	COG0560	E	3.1.3.3		1410957..1411817 [+]	1.95E-06	**1.51**
ape0224	APL_0214	hypothetical protein	COG1518	L		CytoplasmicMembrane	234640..235596 [-]	6.42E-07	**1.51**
cdd	APL_1343	cytidine deaminase	COG0295	F	3.5.4.5		1542869..1543762 [+]	1.18E-07	**1.51**
sdaA	APL_0857	L-serine dehydratase	COG1760	E	4.3.1.17	Cytoplasmic	996347..997726 [+]	5.32E-05	**1.51**
ape1381	APL_1281	hypothetical protein	COG2166	R			1472393..1472785 [+]	3.62E-05	**1.51**
pssA	APL_0043	phosphatidylserine synthase	COG1502	I	2.7.8.8		47008..48372 [-]	6.07E-04	**1.51**
ape2021	APL_1891	hypothetical protein					2123601..2124047 [+]	8.71E-03	**1.50**

G 2 Genes downregulated by ArcA as obtained by microarray analysis

gene	locus tag	gene product	COG #	COG	EC #	psorb loc	location	T-test	ratio
lctP	APL_0447	putative L-lactate permease	COG1620	C		CytoplasmicMembrane	513511..515106 [-]	2.17E-16	**-38.04**
fbp	APL_1450	fructose-1,6-bisphosphatase	COG0158	G	3.1.3.11		1662693..1663697 [+]	8.98E-31	**-25.52**
mqo	APL_1414	putative malate:quinone oxidoreductase	COG0579	R	1.1.99.16		1616829..1618304 [-]	3.55E-10	**-19.89**
aldA	APL_2011	putative aldehyde dehydrogenase aldA	COG1012	C		Cytoplasmic	2236287..2237768 [-]	1.84E-10	**-16.71**
ykgE	APL_0446	putative dehydrogenase subunit	COG0247	C			512616..513341 [-]	1.62E-10	**-15.63**
ompW	APL_1086	outer membrane protein W precursor	COG3047	M		OuterMembrane	1256394..1257041 [-]	2.45E-08	**-12.93**
fdxG	APL_0892	formate dehydrogenase, nitrate-inducible, major subunit	COG0243	C	1.2.1.2	Periplasmic	1033523..1034110 [+]	7.23E-08	**-11.86**
fdxH	APL_0894	formate dehydrogenase, iron-sulfur subunit	COG0437	C		CytoplasmicMembrane	1036582..1037481 [+]	1.17E-05	**-11.21**
fdnI	APL_0895	formate dehydrogenase, cytochrome b556 subunit	COG2864	C	1.2.1.2	CytoplasmicMembrane	1037474..1038145 [+]	2.25E-05	**-11.13**
ape1353	APL_1254	hypothetical protein	COG0471	P		CytoplasmicMembrane	1441994..1443385 [-]	3.03E-08	**-10.23**
fdxG	APL_0893	formate dehydrogenase, nitrate-inducible, major subunit	COG0243	C	1.2.1.2	Periplasmic	1034159..1036582 [+]	5.19E-07	**-10.13**
ykgF	APL_0445	putative electron transport protein	COG1139	C			511197..512606 [-]	1.55E-08	**-9.31**
ape0477	APL_0444	hypothetical protein				Cytoplasmic	510370..511068 [-]	7.91E-08	**-7.39**
yedE	APL_1977	hypothetical protein	COG2391	R		CytoplasmicMembrane	2203946..2205166 [-]	2.96E-03	**-6.84**
lldD	APL_1849	L-lactate dehydrogenase [cytochrome]	COG1304	C			2079893..2081038 [-]	1.14E-06	**-6.41**
dld	APL_0687	D-lactate dehydrogenase	COG0277	C	1.1.1.28		785117..786808 [+]	1.78E-11	**-6.35**
adhI	APL_1959	Alcohol dehydrogenase 1	COG1064	R	1.1.1.1	Cytoplasmic	2189591..2190640 [+]	1.08E-08	**-4.91**
nrdA	APL_0992	ribonucleoside-diphosphate reductase alpha subunit	COG0209	F	1.17.4.1	OuterMembrane	1145469..1147739 [+]	4.77E-04	**-4.74**
maeB	APL_0486	NADP-dependent malic enzyme (NADP-ME)	COG0281	C	1.1.1.40		555322..556590 [+]	1.97E-10	**-4.61**
ape0786	APL_0726	carbonic anhydrase	COG0288	P	4.2.1.1	Cytoplasmic	834060..834776 [+]	3.23E-11	**-4.18**
ape0145	APL_0137	hypothetical protein				CytoplasmicMembrane	138299..139864 [+]	1.06E-11	**-3.80**
ape1523	APL_1416	hypothetical protein					1619045..1619386 [+]	6.37E-06	**-3.75**
yedF	APL_1976	hypothetical protein	COG0425	O			2203659..2203928 [-]	1.28E-02	**-3.57**
ape1797	APL_1680	hypothetical protein	COG3550	R			1905515..1906846 [-]	5.39E-04	**-3.15**
fdhE	APL_0896	formate dehydrogenase accessory protein	COG3058	O			1038255..1039172 [+]	1.14E-05	**-3.13**
lpdA	APL_0771	dihydrolipoyl dehydrogenase	COG1249	C	1.8.1.4	Cytoplasmic	883954..885378 [-]	2.43E-08	**-3.09**
aceE	APL_0773	pyruvate dehydrogenase E1 component	COG2609	C	1.2.4.1		887432..890086 [-]	5.42E-07	**-2.92**
ape1522	APL_1415	hypothetical protein				CytoplasmicMembrane	1618412..1618825 [-]	2.15E-12	**-2.76**
aceF	APL_0772	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)	COG0508	C	2.3.1.12	Cytoplasmic	885474..887373 [-]	3.36E-08	**-2.74**
glpR	APL_0823	glycerol-3-phosphate regulon repressor	COG1349	KG			951400..952161 [-]	4.85E-09	**-2.53**
ape1056	APL_0973	hypothetical protein				CytoplasmicMembrane	1126774..1127511 [-]	1.25E-06	**-2.49**
ape1350	APL_1252	hypothetical protein	COG0471	P		CytoplasmicMembrane	1438796..1440184 [-]	2.11E-10	**-2.46**
mltA	APL_0816	membrane-bound lytic murein transglycolase A precursor			3.2.1.-		942188..943282 [-]	1.54E-03	**-2.43**
ape1054	APL_0971	putative acyl CoA thioester hydrolase	COG1607	I			1125743..1126234 [-]	1.36E-11	**-2.38**
ispZ	APL_0972	putative intracellular septation protein	COG2917	D		CytoplasmicMembrane	1126221..1126772 [-]	3.06E-06	**-2.25**
glnE	APL_0969	glutamate-ammonia-ligase adenylyltransferase	COG1391	OT	2.7.7.42	Cytoplasmic	1122496..1125375 [-]	4.22E-07	**-2.25**
ape1053	APL_0970	hypothetical protein				Cytoplasmic	1125450..1125716 [-]	2.54E-09	**-2.23**
frpB	APL_0276	iron-regulated outer membrane protein B	COG1629	P		OuterMembrane	302893..304863 [+]	3.55E-05	**-2.14**
nrfD	APL_0103	nitrite reductase transmembrane protein	COG3301	P		CytoplasmicMembrane	109216..110184 [+]	1.65E-06	**-2.14**
nrfC	APL_0102	nitrate reductase	COG0437	C			108539..109216 [+]	1.39E-08	**-2.08**
ape0688	APL_0637	hypothetical protein				Cytoplasmic	728268..728729 [+]	9.82E-04	**-2.05**
mglC	APL_1418	galactoside transport system permease protein MglC	COG4211	G		CytoplasmicMembrane	1619924..1620934 [-]	7.41E-05	**-2.03**
fdhD	APL_0891	formate dehydrogenase accessory protein-like	COG1526	C		Cytoplasmic	1032298..1033104 [-]	1.93E-12	**-1.98**
ape2067	APL_1934	hypothetical protein	COG0700			CytoplasmicMembrane	2169338..2169802 [-]	3.84E-07	**-1.97**
ubiA	APL_0822	4-hydroxybenzoate octaprenyltransferase	COG0382	H	2.5.1.-	CytoplasmicMembrane	950519..951400 [-]	3.12E-07	**-1.96**
mutY	APL_1739	A/G-specific adenine glycosylase	COG1194	L	3.2.2.-		1974422..1975567 [+]	2.41E-05	**-1.94**
mscL	APL_1592	large-conductance mechanosensitive channel	COG1970	M		CytoplasmicMembrane	1820008..1820397 [+]	9.47E-07	**-1.94**
nrfE	APL_1052	cytochrome c-type biogenesis protein NrfE	COG1138	O		CytoplasmicMembrane	1220471..1222387 [-]	3.14E-06	**-1.93**

nrfB	APL_0101	cytochrome c-type protein NrfB precursor					107892..108539 [+]	3.77E-07	**-1.92**
nrdB	APL_0993	ribonucleoside-diphosphate reductase subunit beta	COG0208	F	1.17.4.1	Cytoplasmic	1148036..1149166 [+]	7.47E-04	**-1.91**
ape1339	APL_1241	hypothetical protein	COG1699	T		CytoplasmicMembrane	1427864..1429450 [+]	1.80E-09	**-1.91**
yrhG	APL_1902	Hypothetical transport protein yrhG	COG2116	P		CytoplasmicMembrane	2132332..2133024 [+]	5.71E-05	**-1.90**
ape2068	APL_1935	hypothetical protein				CytoplasmicMembrane	2169799..2170224 [-]	4.19E-10	**-1.81**
ape1519	APL_1413	putative long-chain-fatty-acid--CoA ligase	COG1022	I	6.2.1.3	CytoplasmicMembrane	1614873..1616648 [-]	6.84E-04	**-1.80**
ccmA	APL_1372	cytochrome c biogenesis ATP-binding export protein CcmA	COG4133	O		CytoplasmicMembrane	1568850..1569485 [-]	2.07E-05	**-1.79**
ape1524	APL_1417	hypothetical protein				CytoplasmicMembrane	1619428..1619853 [+]	5.80E-03	**-1.78**
ilvM	APL_0098	Acetolactate synthase isozyme II small subunit (AHAS-II)			2.2.1.6		104072..104290 [-]	1.17E-04	**-1.76**
cyaA	APL_1054	adenylate cyclase	COG3072	F	4.6.1.1	Cytoplasmic	1224512..1227040 [-]	6.73E-09	**-1.76**
ansB	APL_0135	probable L-asparaginase periplasmic precursor	COG0252	EJ	3.5.1.1	Periplasmic	149916..150965 [-]	5.56E-06	**-1.76**
gltS	APL_0967	sodium/glutamate symporte carrier protein	COG0786	E		CytoplasmicMembrane	1120735..1121952 [+]	1.91E-06	**-1.75**
nrfA	APL_0100	Cytochrome c-552 precursor	COG3303	P	1.7.2.2	Periplasmic	106390..107871 [+]	4.45E-04	**-1.74**
ape0961	APL_0890	hypothetical protein	COG0679	R		CytoplasmicMembrane	1031364..1032296 [-]	9.95E-04	**-1.74**
rplW	APL_1762	50S ribosomal protein L23	COG0089	J			1998351..1998656 [+]	6.28E-06	**-1.74**
ccmB	APL_1371	heme exporter protein B	COG2386	O		CytoplasmicMembrane	1568191..1568853 [-]	4.55E-05	**-1.73**
icc	APL_1942	protein Icc-like	COG1409	R		Cytoplasmic	2174466..2175293 [-]	3.56E-06	**-1.72**
ccmC	APL_1370	heme exporter protein C	COG0755	O		CytoplasmicMembrane	1567409..1568140 [-]	1.89E-05	**-1.71**
cydA	APL_0297	cytochrome oxidase subunit 1	COG1271	C	1.10.3.-	CytoplasmicMembrane	329378..330925 [+]	5.42E-05	**-1.71**
tldD	APL_1540	TldD-like protein	COG0312	R		Cytoplasmic	1753394..1754854 [-]	1.90E-04	**-1.71**
ape1960	APL_1832	hypothetical protein	COG2041	R			2055320..2056276 [+]	1.33E-04	**-1.70**
ape1961	APL_1833	hypothetical protein	COG2717			CytoplasmicMembrane	2056276..2056869 [+]	4.17E-05	**-1.70**
ulaA	APL_1700	predicted ascorbate-specific permease IIC component			2.7.1.69	CytoplasmicMembrane	1931109..1932890 [-]	1.93E-05	**-1.69**
aqpZ	APL_1457	aquaporin Z	COG0580	G		CytoplasmicMembrane	1671115..1671801 [-]	5.11E-06	**-1.69**
cydB	APL_0298	cytochrome oxidase subunit 2	COG1294	C	1.10.3.-	CytoplasmicMembrane	330942..332078 [+]	3.43E-08	**-1.68**
ape1946	APL_1818	hypothetical protein	COG1559	R			2040329..2041363 [-]	3.84E-04	**-1.67**
kefBC	APL_1053	glutathione-regulated potassium-efflux system protein	COG0475	P		CytoplasmicMembrane	1222558..1224423 [-]	1.62E-09	**-1.66**
nhaA	APL_1947	Na+/H+ antiporter 1 (Sodium/proton antiporter 1)	COG3004	P		CytoplasmicMembrane	2177937..2179121 [+]	1.13E-06	**-1.65**
ftsJ	APL_0594	ribosomal RNA large subunit methyltransferase J	COG0293	J	2.1.1.-		678772..679398 [+]	6.17E-06	**-1.65**
ape0917	APL_0848	putative ABC transporter periplasmic binding protein	COG0747	E		CytoplasmicMembrane	983413..984987 [+]	3.47E-06	**-1.64**
ccmF	APL_1367	cytochrome c-type biogenesis protein CcmF	COG1138	O		CytoplasmicMembrane	1564521..1566470 [-]	5.30E-06	**-1.63**
napF	APL_1431	ferredoxin-type protein NapF	COG1145	C		Cytoplasmic	1636794..1637324 [-]	1.08E-06	**-1.63**
rplD	APL_1761	50S ribosomal protein L4	COG0088	J			1997752..1998354 [+]	9.90E-09	**-1.63**
ape0062	APL_0060	putative N6-adenine-specific DNA methylase	COG0116	L			65092..67230 [+]	5.10E-06	**-1.63**
ape2066	APL_1933	acetylornithine deacetylase/succinyl-diaminopimelate desuccinylase and related deacylases	COG0624	E		Cytoplasmic	2168701..2169324 [-]	1.43E-05	**-1.62**
rpsJ	APL_1759	30S ribosomal protein S10	COG0051	J			1996773..1997084 [+]	3.51E-06	**-1.62**
pckA	APL_0800	phosphoenolpyruvate carboxykinase [ATP]	COG1866	C	4.1.1.49	Cytoplasmic	922121..923731 [-]	6.20E-04	**-1.61**
ape1478	APL_1373	hypothetical protein	COG0593	L		Cytoplasmic	1569626..1570336 [-]	6.15E-04	**-1.60**
lipA	APL_1593	lipoyl synthase	COG0320	H	2.8.1.-	Cytoplasmic	1820527..1821519 [-]	2.41E-04	**-1.59**
rplC	APL_1760	50S ribosomal protein L3	COG0087	J			1997110..1997736 [+]	3.88E-05	**-1.59**
ape0051	APL_0049	hypothetical protein				OuterMembrane	55612..56130 [+]	1.38E-09	**-1.59**
hflK	APL_1077	protein HflK	COG0330	O	3.4.-.-		1245823..1247013 [-]	1.26E-05	**-1.58**
chuW	APL_1523	coproporphyrinogen III oxidase	COG0635	H	1.3.99.22	Cytoplasmic	1736466..1738283 [-]	1.58E-04	**-1.58**
cysZ	APL_1304	putative sulfate transport protein CysZ	COG2981	E		CytoplasmicMembrane	1495357..1496238 [+]	9.28E-06	**-1.57**
ape0148	APL_0140	hypothetical protein	COG3835	KT		Cytoplasmic	155925..157040 [+]	1.82E-05	**-1.57**
galK	APL_0995	galactokinase	COG0153	G	2.7.1.6	Cytoplasmic	1150505..1151659 [+]	9.42E-04	**-1.56**
pgaC	APL_1923	biofilm PGA synthesis N-glycosyltransferase PgaC	COG1215	M		CytoplasmicMembrane	2160319..2161554 [+]	2.05E-02	**-1.56**
rpe	APL_1820	ribulose-phosphate 3-epimerase	COG0036	G	5.1.3.1	Cytolasmic	2042459..2043133 [-]	8.34E-06	**-1.56**
ligA	APL_1302	DNA ligase	COG0272	L	6.5.1.2	Cytoplasmic	1492112..1494163 [-]	1.04E-04	**-1.55**
afuB	APL_1447	Ferric transport system permease protein fpbB	COG1178	P		CytoplasmicMembrane	1657959..1660022 [+]	1.14E-03	**-1.54**

holB	APL_1816	DNA polymerase III subunit delta	COG0470	L	2.7.7.7	Cytplasmic	2038713..2039696 [-]	8.37E-04	**-1.54**
metC	APL_0320	cystathionine beta-lyase	COG0626	E	4.4.1.8		356847..358037 [-]	5.77E-05	**-1.54**
surE	APL_1927	5'-nucleotidase surE	COG0496	R	3.1.3.5	Cytoplasmic	2164002..2164766 [+]	4.28E-05	**-1.54**
ape1343	APL_1245	hypothetical protein				CytoplasmicMembrane	1432923..1433441 [-]	9.19E-07	**-1.53**
afuA	APL_1446	ABC-type Fe3+ transport system, periplasmic component	COG1840	P		Periplasmic	1656814..1657854 [+]	9.16E-07	**-1.52**
ape0969	APL_0898	putative oxalate/formate antiporter	COG0477	GEPR		CytoplasmicMembrane	1040777..1042325 [+]	2.08E-04	**-1.52**
ccmE	APL_1368	cytochrome c-type biogenesis protein CcmE	COG2332	O			1566470..1567015 [-]	1.74E-03	**-1.51**
putA	APL_0106	bifunctional protein PutA [Includes: Proline dehydrogenase; Delta-1-pyrroline-5-carboxylat dehydrogenase]	COG4230	C	1.5.1.12 and 1.5.99.8	Cytoplasmic	121566..125171 [-]	1.67E-05	**-1.50**

G 3 Genes upregulated by HlyX as obtained by microarray analysis

gene	locus_tag	gene product	COG #	COG	EC #	psorb_loc	location	T-test	ratio
ape0761	APL_0705	putative methylation subunit, type III restriction-modification system	COG2189	L	2.1.1.72	OuterMembrane	813144..816032 [-]	8.52E-08	**29.81**
hlyX	APL_0656	regulatory protein HlyX	COG0664	T		Cytoplasmic	750742..751509 [+]	2.26E-07	**26.35**
dmsA	APL_1674	anaerobic dimethyl sulfoxide reductase chain A precursor	COG0243	C	1.8.99.-	Periplasmic	1900092..1902509 [+]	1.06E-07	**25.35**
nrfA	APL_0100	Cytochrome c-552 precursor	COG3303	P	1.7.2.2	Periplasmic	106390..107871 [+]	1.93E-06	**21.62**
hyaA	APL_1331	hydrogenase-2 small chain precursor	COG1740	C	1.12.99.6	Periplasmic	1527737..1528879 [+]	1.53E-05	**20.42**
ccp	APL_1379	cytochrome c peroxidase	COG1858	P	1.11.1.5	Periplasmic	1581367..1582767 [+]	8.25E-07	**20.36**
torY	APL_0689	cytochrome c-type protein TorY	COG3005	C		CytoplasmicMembrane	789616..790734 [-]	1.44E-10	**17.61**
napA	APL_1429	periplasmic nitrate reductase precursor	COG0243	C	1.7.99.4	Periplasmic	1634007..1636490 [-]	9.73E-08	**14.93**
napG	APL_1428	ferredoxin-type protein napG-like	COG1145	C		Cytoplasmic	1632967..1633872 [-]	4.79E-08	**14.79**
hybB	APL_1333	putative Ni/Fe-hydrogenase 2 b-type cytochrome subunit	COG5557	C		CytoplasmicMembrane	1529902..1531083 [+]	3.10E-08	**12.71**
napH	APL_1427	ferredoxin-type protein NapH-like	COG034	C		CytoplasmicMembrane	1632074..1632967 [-]	1.82E-08	**12.58**
napC	APL_1425	cytochrome c-type protein NapC	COG3005	C			1631007..1631621 [-]	9.44E-08	**12.31**
ape1539	APL_1432	putative NAD(P)H oxidoreductase	COG2249	R	1.6.99.-		1637523..1638098 [+]	1.05E-05	**12.19**
hybA	APL_1332	hydrogenase-2 operon protein HybA precursor	COG0437	C		Periplasmic	1528872..1529909 [+]	6.55E-09	**11.67**
torZ	APL_0688	trimethylamine-N-oxide reductase precursor	COG0243	C	1.7.2.3	Periplasmic	786946..789420 [-]	9.76E-09	**11.07**
hyaB	APL_1334	hydrogenase-2 large chain precursor	COG0374	C	1.12.99.6	Periplasmic	1531080..1532789 [+]	2.30E-06	**10.50**
napB	APL_1426	nitrate reductase cytochrome c-type subunit	COG3043	C		Periplasmic	1631633..1632055 [-]	4.60E-13	**10.18**
ape0760	APL_0704	hypothetical protein					809428..812742 [-]	8.96E-11	**9.56**
ape2089	APL_1955	outer membrane receptor proteins, mostly Fe transport	COG1629	P		OuterMembrane	2184211..2184735 [+]	9.89E-05	**9.42**
tonB2	APL_0076	protein TonB2	COG0810	M		CytoplasmicMembrane	86901..87767 [-]	7.11E-08	**9.27**
dmsC	APL_1676	anaerobic dimethyl sulfoxide reductase chain C	COG3302	R		CytoplasmicMembrane	1903138..1903971 [+]	1.67E-15	**8.67**
ape2087	APL_1953	outer membrane receptor proteins, mostly Fe transport	COG1629	P		OuterMembrane	2182851..2183384 [+]	3.01E-05	**8.36**
nrfB	APL_0101	cytochrome c-type protein NrfB precursor					107892..108539 [+]	3.93E-07	**8.35**
hcr	APL_1547	NADH oxidoreductase	COG1018	C			1769175..1770194 [+]	1.52E-07	**8.05**
ape2088	APL_1954	outer membrane receptor proteins, mostly Fe transport	COG1629	P			2183478..2184122 [+]	2.50E-04	**8.04**
ape0758	APL_0703	hypothetical ATP-dependent helicase	COG0553	KL		Cytoplasmic	806555..809425 [-]	2.00E-07	**7.96**
adh2	APL_1011	aldehyde-alcohol dehydrogenase 2 [Includes: Alcohol dehydrogenase; Acetaldehyde dehydrogenase]	COG1454	C	1.1.1.1 1.2.1.10	Cytoplasmic	1175244..1177859 [+]	6.73E-07	**7.87**
dmsB	APL_1675	anaerobic dimethyl sulfoxide reductase chain B	COG0437	C		CytoplasmicMembrane	1902519..1903136 [+]	2.48E-06	**7.55**
hlpB	APL_0332	lipoprotein HlpB					368623..369102 [-]	6.59E-07	**7.26**
nrfC	APL_0102	nitrate reductase	COG0437	C			108539..109216 [+]	3.19E-06	**7.10**
napF	APL_1431	ferredoxin-type protein NapF	COG1145	C		Cytoplasmic	1636794..1637324 [-]	7.01E-06	**6.89**
ape0031	APL_0029	ABC transporter periplasmic protein	COG0747	E		Periplasmic	33833..35407 [-]	5.88E-10	**6.67**
visC	APL_0333	putative monooxygenase family protein	COG0654	HC			369238..370383 [-]	2.60E-08	**6.26**
ape2004	APL_1875	hypothetical protein					2103986..2104696 [+]	3.78E-13	**6.07**
ape2007	APL_1878	hypothetical protein				OuterMembrane	2106838..2107857 [+]	7.69E-11	**5.90**
ape2168	APL_2031	Hypothetical protein	COG0526			Cytoplasmic	2257942..2258436 [-]	1.47E-06	**5.73**
napD	APL_1430	putative napD protein	COG3062	P		Cytoplasmic	1636522..1636791 [-]	3.11E-05	**5.68**
ape0312	APL_0296	hypothetical protein					328590..328922 [-]	2.41E-06	**5.67**
nrfD	APL_0103	nitrite reductase transmembrane protein	COG3301	P		CytoplasmicMembrane	109216..110184 [+]	2.19E-10	**5.65**
htpG	APL_0987	chaperone protein HtpG	COG0326	O		Cytoplasmic	1139729..1141606 [-]	1.03E-06	**5.28**
ape0354	APL_0334	hypothetical protein	COG1660	R		Cytoplasmic	370507..371367 [-]	2.73E-07	**5.17**
ape2008	APL_1879	hypothetical protein	COG1629				2107994..2109331 [+]	7.45E-08	**5.12**
ape0491	APL_0457	hypothetical metalloprotease	COG0739	M			527966..529456 [+]	1.72E-05	**4.90**
ape1690	APL_1575	hypothetical protein					1801954..1802508 [-]	3.61E-11	**4.89**
ape2086	APL_1952	outer membrane receptor proteins, mostly Fe transport	COG1629	P		OuterMembrane	2182535..2182882 [+]	6.71E-05	**4.70**

ape1779	APL_1662	PTS system mannose-specific EIIAB component [Includes: Mannose-specific phosphotransferase enzyme IIA component; Mannose-specific phosphotransferase enzyme IIB component]	COG3444	G	2.7.1.69	Cytoplasmic	1888149..1889117 [-]	3.19E-09	**4.52**
exbD2	APL_0077	biopolymer transport protein ExbD2	COG0848	U		CytoplasmicMembrane	87777..88166 [-]	2.00E-07	**4.37**
ape0277	APL_0264	putative ABC transporter ATP-binding protein	COG1131	V		CytoplasmicMembrane	292788..293696 [-]	6.32E-07	**4.33**
ape2021	APL_1891	hypothetical protein					2123601..2124047 [+]	2.57E-06	**4.30**
ape0436	APL_0410	hypothetical outer membrane protein	COG2825	M		OuterMembrane	466705..467511 [-]	3.65E-08	**4.28**
hlp	APL_0331	putative lipoprotein					367865..368542 [-]	1.50E-07	**4.26**
ape0972	APL_0901	ribosome-associated inhibitor A	COG1544	J		Cytoplasmic	1044565..1044879 [+]	8.82E-07	**4.13**
exbB2	APL_0078	biopolymer transport protein ExbB2	COG0811	U			88211..88663 [-]	9.77E-06	**4.11**
ape1248	APL_1157	putative malate transporter 5'-end	COG0471	P		CytoplasmicMembrane	1333969..1334664 [-]	5.12E-05	**4.08**
ssa1	APL_0364	serotype-specific antigen 1 precursor	COG1404	O		OuterMembrane	404826..407621 [-]	1.08E-05	**4.02**
hcp	APL_1546	hydroxylamine reductase	COG1151	C	1.7.-.-	Cytoplasmic	1767397..1769052 [+]	5.77E-07	**3.97**
lon	APL_0376	ATP-dependent protease La	COG0466	O	3.4.21.53	Cytoplasmic	425663..428071 [-]	1.06E-06	**3.97**
tmk	APL_1817	thymidylate kinase	COG0125	F	2.7.4.9	Cytoplasmic	2039699..2040325 [-]	3.69E-07	**3.96**
ape1759	APL_1643	hypothetical protein	COG2923	P			1866721..1867092 [+]	5.72E-07	**3.94**
ape2179	APL_2041	Hypothetical protein					2268504..2269370 [-]	6.24E-07	**3.92**
dnaK	APL_1906	Chaperone protein dnaK	OG0443	O			2136557..2138455 [-]	2.19E-05	**3.90**
lipB	APL_1594	lipoyltransferase	COG0321	H	2.3.1.-	Cytoplasmic	1821631..1822287 [-]	2.05E-06	**3.89**
ape1059	APL_0975	hypothetical protein	COG0037	D		Cytoplasmic	1128272..1129228 [+]	1.25E-04	**3.88**
ape1390	APL_1289	hypothetical protein	COG2854	Q			1479067..1479705 [-]	3.90E-12	**3.86**
dsbC	APL_0458	thiol:disulfide interchange protein DsbC precursor	COG1651	O	5.3.4.1	Periplasmic	529475..530170 [+]	2.08E-09	**3.85**
ape1794	APL_1677	hypothetical protein	COG3381	R			1904027..1904632 [+]	2.14E-11	**3.84**
alr	APL_0252	alanine racemase	COG0787	M	5.1.1.1	Cytoplasmic	274912..276036 [-]	3.20E-10	**3.81**
lipA	APL_1593	lipoyl synthase	COG0320	H	2.8.1.-	Cytoplasmic	1820527..1821519 [-]	8.13E-07	**3.73**
ptsN	APL_0335	PTS system, nitrogen regulatory IIA-like protein	COG1762	GT	2.7.1.69	Cytoplasmic	371386..371898 [-]	3.45E-08	**3.72**
tolA	APL_0302	cell envelope integrity inner membrane protein TolA	COG3064	M			334285..335580 [+]	3.88E-06	**3.69**
sdaC	APL_0856	serine transporter	COG0814	E		CytoplasmicMembrane	995022..996251 [+]	1.39E-05	**3.65**
ape0215	APL_0205	predicted rRNA methyltransferase	COG0566	J	2.1.1.-		223489..224526 [-]	8.94E-11	**3.65**
ape1959	APL_1831	DNA-directed RNA polymerase specialized sigma subunit, sigma-24 like	COG1595	K		Cytoplasmic	2054650..2055231 [-]	5.21E-13	**3.65**
tusD	APL_1642	sulfurtransferase TusD-like	COG1553	P			1866341..1866718 [+]	1.36E-10	**3.62**
ubiG	APL_0285	3-demethylubiquinone-9 3-methyltransferase	COG2227	H	2.1.1.64		314720..315424 [-]	1.49E-10	**3.62**
macA	APL_1814	probable macrolide-specific efflux protein	COG0845	M			2036534..2037310 [-]	2.63E-14	**3.57**
yhgB	APL_0336	putative ABC transporter ATP-binding protein	COG1137	R		Cytoplasmic	371948..372673 [-]	6.88E-10	**3.51**
ape0358	APL_0338	hypothetical protein					373211..373807 [-]	6.64E-08	**3.50**
kdsA	APL_1578	2-dehydro-3-deoxyphosphooctonate aldolase	COG2877	M	2.5.1.55	Cytoplasmic	1804277..1805137 [-]	9.54E-08	**3.44**
ape0357	APL_0337	hypothetical protein					372683..373186 [-]	1.27E-07	**3.40**
ape1712	APL_1597	possible rare lipoprotein A RlpA-like protein	COG0797	M			1824026..1824589 [-]	1.24E-08	**3.40**
dacA	APL_1596	D-alanyl-D-alanine carboxypeptidase fraction A	COG1686	M	3.4.16.4	CytoplasmicMembrane	1822752..1823930 [-]	3.46E-06	**3.38**
pssA	APL_0043	phosphatidylserine synthase	COG1502	I	2.7.8.8		47008..48372 [-]	7.81E-07	**3.35**
uspA	APL_0655	universal stress protein A-like	COG0589	T		Cytoplasmic	750053..750478 [-]	9.60E-06	**3.35**
nadR	APL_0046	transcriptional regulator NadR	COG3172	H	2.7.7.1	Cytoplasmic	52696..53976 [+]	5.44E-09	**3.34**
rseA	APL_0395	putative sigma-E factor negative regulatory protein	COG3073	T			449684..450307 [+]	6.72E-10	**3.34**
exbD	APL_1569	biopolymer transport ExbD protein	COG0848	U		CytoplasmicMembrane	1797580..1797990 [-]	8.84E-12	**3.31**
hypB	APL_1327	hydrogenase isoenzymes nickel incorporation protein HypB	COG0378	OK		Cytoplasmic	1522186..1522995 [+]	8.19E-07	**3.24**
ape1065	APL_0981	hypothetical protein	COG0859	M			1134486..1135547 [+]	3.79E-10	**3.20**
exbB	APL_1570	biopolymer transport ExbB protein	COG0811	U		CytoplasmicMembrane	1797990..1798658 [-]	1.47E-06	**3.15**
ape1324	APL_1227	hypothetical protein				Cytoplasmic	1408957..1409745 [+]	1.94E-06	**3.11**
recA	APL_1143	recombinase A	COG0468	L		Cytoplasmic	1318766..1319896 [-]	6.11E-07	**3.10**

kdsA	APL_2040	2-dehydro-3-deoxyphosphooctonate aldolase	COG2877	M	2.5.1.55	Cytoplasmic	2267599..2268453 [-]	6.84E-07	**3.06**
holB	APL_1816	DNA polymerase III subunit delta	COG0470	L	2.7.7.7	Cytoplasmic	2038713..2039696 [-]	1.56E-07	**3.04**
ape0776	APL_0717	iron(III) ABC transporter, ATP-binding protein	COG4604	P	3.6.3.34		827141..827899 [	4.89E-09	**3.02**
ape0142	APL_0134	hypothetical protein					149191..149835 [+]	3.07E-07	**3.02**
ilvM	APL_0098	Acetolactate synthase isozyme II small subunit (AHAS-II)			2.2.1.6	Cytoplasmic	104072..104290 [-]	1.15E-05	**3.02**
oapA	APL_1405	opacity associated protein A	COG3061	M		Extracellular	1605788..1607179 [-]	7.34E-07	**3.02**
grcA	APL_0361	autonomous glycyl radical cofactor	COG3445	R		Cytoplasmic	402489..402875 [-]	1.95E-08	**2.99**
ape1965	APL_1836	hypothetical protein					2059991..2060503 [+]	1.02E-07	**2.94**
rluE	APL_0088	ribosomal large subunit pseudouridine synthase E	COG1187	J	5.4.99.12	Cytoplasmic	95649..96344 [-]	7.52E-07	**2.93**
ape1342	APL_1244	hypothetical protein				Cytoplasmic	1432223..1432834 [+]	4.14E-09	**2.91**
ape1502	APL_1397	hypothetical protein				Cytoplasmic	1597474..1598262 [-]	8.51E-05	**2.91**
ape0701	APL_0650	hypothetical protein				Cytoplasmic	743985..744503 [+]	7.72E-11	**2.91**
ape2035	APL_1904	hypothetical protein				Cytoplasmic	2134138..2134926 [-]	2.05E-04	**2.90**
hrpA	APL_0365	ATP-dependent RNA helicase	COG1643	L	3.6.1.-		407842..411741 [-]	3.86E-07	**2.89**
clpB	APL_1087	chaperone ClpB	COG0542	O		Cytoplasmic	1257302..1259875 [+]	1.19E-03	**2.89**
ape0416	APL_0392	hypothetical protein	COG0561	R		Cytoplasmic	445790..446605 [-]	5.35E-06	**2.87**
ape1247	APL_1156	putative malate transporter 3'-end	COG0471	P		CytoplasmicMembrane	1333204..1333872 [-]	2.16E-04	**2.86**
ape0384	APL_0363	hypothetical protein					403856..404728 [-]	1.01E-03	**2.85**
ape0132	APL_0124	hypothetical protein	COG0510	M			141240..141989 [-]	1.94E-09	**2.85**
ape1150	APL_1061	hypothetical protein					1230590..1231186 [+]	2.43E-05	**2.83**
engB	APL_0094	putative GTP-binding protein EngB	COG0218	R			99769..100389 [-]	8.23E-06	**2.83**
ape0311	APL_0295	hypothetical protein	COG1179	H			327747..328511 [+]	4.39E-12	**2.82**
ldhA	APL_2039	Glycerate dehydrogenase	COG1052	CHR		Cytoplasmic	2266593..2267543 [-]	2.02E-06	**2.81**
cpxR	APL_0629	transcriptional regulatory protein CpxR	COG0745	TK		Cytoplasmic	716776..717504 [-]	2.26E-09	**2.81**
ape1558	APL_1451	hypothetical protein				Cytoplasmic	1663853..1664464 [-]	2.20E-04	**2.81**
hslU	APL_1735	ATP-dependent hsl protease ATP-binding subunit hslU	COG1220	O		Cytoplasmic	1970984..1972306 [-]	2.14E-06	**2.80**
narP	APL_0059	nitrate/nitrite response regulator protein	COG2197	TK		Cytoplasmic	64259..64888 [-]	1.18E-08	**2.79**
ape0099	APL_0096	zinc transporter family protein ZIP	COG0428	P		CytoplasmicMembrane	101138..101968 [-]	1.30E-07	**2.79**
sdaA	APL_0857	L-serine dehydratase	COG1760	E	4.3.1.17	Cytoplasmic	996347..997726 [+]	4.54E-06	**2.79**
macA	APL_0391	probable macrolide-specific efflux protein	COG0845	M		CytoplasmicMembrane	444512..445693 [-]	1.17E-06	**2.79**
gcvA	APL_0131	glycine cleavage system transcriptional activator-like	COG0583	K		Cytoplasmic	146637..147527 [+]	3.60E-08	**2.78**
sanA	APL_0442	SanA protein				CytoplasmicMembrane	499069..499755 [+]	1.92E-13	**2.77**
hisI	APL_2028	Histidine biosynthesis bifunctional protein hisIE [Includes: Phosphoribosyl-AMP cyclohydrolase (PRA-CH); Phosphoribosyl-ATP pyrophosphatase (PRA-PH)]	COG0139	E	3.5.4.19 3.6.1.31	Cytoplasmic	2255532..2256176 [+]	1.67E-05	**2.73**
grxA	APL_0054	glutaredoxin	COG0695	O		Cytoplasmic	60795..61058 [+]	1.67E-06	**2.71**
tonB1	APL_1571	periplasmic protein	COG0810	M			1798680..1799420 [-]	1.30E-05	**2.71**
prc	APL_0120	tail-specific protease precursor	COG0793	M	3.4.21.102	Cytoplasmic	136190..138202 [+]	9.17E-06	**2.70**
ape1778	APL_1661	mannose permease IIC component	COG3715	G		CytoplasmicMembrane	1887340..1888134 [-]	1.71E-04	**2.69**
sygB	APL_1803	Glycyl-tRNA synthetase beta chain	COG0751	J	6.1.1.14		2025782..2027851 [+]	1.42E-11	**2.68**
tbpA	APL_1567	transferrin-binding protein 1 Tbp1	COG1629	P		OuterMembrane	1793088..1795895 [-]	1.43E-07	**2.68**
ape0309	APL_0293	putative type I site-specific restriction-modification system, R (restriction) subunit	COG0610	V			322648..325785 [+]	6.61E-13	**2.68**
ape0308	APL_0292	hypothetical protein					322055..322642 [+]	1.65E-07	**2.68**
ape1626	APL_1516	cytochrome c biogenesis factor-like	COG4235	O			1732016..1732930 [+]	5.16E-07	**2.67**
mglB	APL_0450	D-galactose-binding periplasmic protein precursor	COG1879	G		Periplasmic	518260..519231 [+]	9.28E-05	**2.67**
typA	APL_0053	GTP-binding protein	COG1217	T		Cytoplasmic	58930..60636 [+]	1.30E-05	**2.67**
pheA	APL_1033	P-protein [Includes: Chorismate mutase; Prephenate dehydratase]	COG0077	E	4.2.1.51 5.4.99.5	Cytoplasmic	1198612..1199769 [-]	5.78E-08	**2.65**
hypD	APL_1328	hydrogenase isoenzymes formation protein HypD	COG0409	O		Cytoplasmic	1523007..1524122 [+]	9.50E-08	**2.65**
ape2139	APL_2002	Hypothetical protein	COG0457			OuterMembrane	2226751..2228220 [-]	1.46E-08	**2.62**
ape0049	APL_0047	hypothetical diadenosine tetraphosphatase	COG0639	T			53973..54671 [+]	4.67E-06	**2.62**

ape1757	APL_1641	hypothetical protein					1865682..1866341 [+]	4.74E-06	**2.61**
ape1710	APL_1595	hypothetical protein				Cytoplasmic	1822319..1822618 [-]	6.51E-05	**2.61**
frpB	APL_0276	iron-regulated outer membrane protein B	COG1629	P		OuterMembrane	302893..304863 [+]	3.96E-05	**2.60**
serC	APL_0702	phosphoserine aminotransferase	COG1932	HE		Cytoplasmic	805273..806361 [-]	2.02E-07	**2.59**
sirA	APL_0092	sulfurtransferase	COG0425	O		Cytoplasmic	99038..99277 [+]	4.91E-05	**2.59**
hypF	APL_1330	carbamoyltransferase HypF	COG0068	O			1525217..1527502 [-]	3.56E-06	**2.57**
ape1151	APL_1062	hypothetical protein					1231227..1231601 [+]	1.39E-03	**2.57**
lpxA	APL_0407	Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase	COG1043	M	2.3.1.129	Cytoplasmic	464325..465119 [-]	5.38E-12	**2.57**
ape1854	APL_1729	hypothetical protein				OuterMembrane	1965725..1967146 [+]	1.90E-07	**2.56**
ape0350	APL_0330	hypothetical protein					367332..367808 [-]	1.80E-06	**2.55**
ape1930	APL_1802	hypothetical protein					2025340..2025744 [+]	1.17E-04	**2.53**
ape1548	APL_1441	hypothetical protein				Cytoplasmic	1648236..1648847 [-]	8.24E-05	**2.52**
ape0134	APL_0126	HIT-like protein	COG0537	FGR		Cytoplasmic	142362..142727 [-]	3.11E-07	**2.52**
ape1138	APL_1049	hypothetical protein	COG4235	O			1218778..1219524 [-]	1.61E-06	**2.52**
ung	APL_0362	uracil-DNA glycosylase	COG0692	L	3.2.2.-		403081..403758 [+]	2.18E-04	**2.51**
surA	APL_0400	peptidyl-prolyl cis-trans isomerase SurA	COG0760	O	5.2.1.8	Periplasmic	454398..455348 [-]	1.22E-04	**2.50**
mazG	APL_0630	predicted pyrophosphatase	COG1694	R		Cytoplasmic	717629..718228 [-]	2.15E-07	**2.50**
ape1946	APL_1818	hypothetical protein	COG1559	R			2040329..2041363 [-]	1.76E-05	**2.49**
apxID	APL_1442	RTX-I toxin secretion component	COG0845	M			1649278..1650714 [-]	5.15E-10	**2.48**
ape1943	APL_1815	hypothetical protein	COG3713	M		OuterMembrane	2037606..2038382 [-]	1.46E-07	**2.47**
fis	APL_0190	DNA-binding protein Fis	COG2901	KL			210047..210343 [+]	1.64E-04	**2.46**
ape0140	APL_0132	putative haloacid dehalogenase-like hydrolase	COG1011	R			147527..148207 [+]	1.90E-09	**2.45**
ileS	APL_0044	isoleucyl-tRNA synthetase	COG0060	J	6.1.1.5	Cytoplasmic	48524..51340 [-]	4.84E-14	**2.41**
valS	APL_1502	valyl-tRNA synthetase	COG0525	J	6.1.1.9	Cytoplasmic	1717631..1720495 [-]	6.07E-08	**2.41**
infA	APL_1228	translation initiation factor IF-1	COG0361	J		Cytoplasmic	1409885..1410103 [-]	5.32E-04	**2.41**
lolC	APL_2038	Lipoprotein-releasing system transmembrane protein lolC	COG4591	M		CytoplasmicMembrane	2265296..2266468 [-]	7.66E-06	**2.39**
ape1514	APL_1409	predicted CueR-like transcriptional regulator	COG0789	K		Cytoplasmic	1611389..1612009 [-]	9.28E-05	**2.38**
ape0096	APL_0093	hypothetical protein				Cytoplasmic	99327..99767 [-]	2.12E-05	**2.38**
fumC	APL_1757	Fumarate hydratase class II	COG0114	C	4.2.1.2	Cytoplasmic	1994160..1995554 [	1.84E-08	**2.38**
oapB	APL_1404	opacity associated protein B					1605262..1605666 [-]	1.36E-09	**2.38**
ape0272	APL_0259	predicted oligoketide cyclase/lipid transport protein	COG2867	I			288879..289313 [-]	6.66E-05	**2.38**
hgbA	APL_1047	hemoglobin-binding protein A precursor	COG1629	P		OuterMembrane	1215150..1217993 [-]	2.55E-04	**2.35**
ape1760	APL_1644	hypothetical protein	COG2168	P			1867105..1867374 [+]	5.86E-04	**2.35**
cpxC	APL_1583	capsule polysaccharide export inner-membrane protein	COG3524	M			1810436..1811593 [+]	3.64E-07	**2.35**
hslV	APL_1736	ATP-dependent protease hslV	COG5405	O	3.4.25.-	Cytoplasmic	1972361..1972882 [-]	7.93E-06	**2.34**
era	APL_0544	GTP-binding protein Era-like	COG1159	R		Cytoplasmic	614750..615664 [+]	9.85E-08	**2.34**
ape1938	APL_1810	Putative Mg-dependent DNAse	COG0084	L	3.1.21.-		2031836..2032624 [-]	6.49E-07	**2.32**
ape2181	APL_2043	hypothetical protein					2270245..2270772 [-]	3.82E-09	**2.30**
ape1239	APL_1148	putative periplasmic protein					1324003..1324452 [+]	1.12E-04	**2.30**
lpxD	APL_0409	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	COG1044	M	2.3.1.-	Cytoplasmic	465680..466705 [-]	4.15E-08	**2.30**
yhbZ	APL_0040	hypothetical GTP-binding protein	COG0536	R		Cytoplasmic	44225..45400 [+]	1.02E-05	**2.30**
hypE	APL_1329	hydrogenase isoenzymes formation protein HypE	COG0309	O		Cytoplasmic	1524124..1525137 [+]	4.27E-05	**2.29**
nudC	APL_0087	NADH pyrophosphatase	COG2816	L	3.6.1.-	Cytoplasmic	94861..95631 [-]	3.15E-04	**2.28**
ape1385	APL_1285	hypothetical protein	COG0607	P			1476170..1476538 [+]	9.61E-04	**2.28**
recJ	APL_0459	single-stranded-DNA-specific exonuclease Rec	COG0608	L	3.1.-.-	Cytoplasmic	530220..531977 [+]	7.96E-05	**2.28**
htrA	APL_1293	probable periplasmic serine protease do/hhoA-like precursor	COG0265	O	3.4.21.-	Periplasmic	1482162..1483556 [+]	9.89E-05	**2.28**
bioD1	APL_0614	dethiobiotin synthetase 1	COG0132	H	6.3.3.3		700088..700807 [-]	4.75E-09	**2.28**
ape0641	APL_0590	hypothetical protein	COG3955	M			672412..673032 [-]	2.85E-07	**2.27**
ape0121	APL_0113	hypothetical protein					131234..131824 [+]	1.01E-06	**2.26**

hemK	APL_2042	protein HemK-like	COG2890	J	2.1.1.-		2269371..2270243 [-]	1.09E-06	**2.26**
cysB	APL_0133	HTH-type transcriptional regulator CysB	COG0583	K		Cytoplasmic	148207..149190 [+]	2.13E-06	**2.26**
rsmB	APL_1560	ribosomal RNA small subunit methyltransferase B	COG0144	J	2.1.1.-	Cytoplasmic	1784135..1785433 [-]	7.24E-05	**2.25**
ape1859	APL_1734	hypothetical protein				CytoplasmicMembrane	1970132..1970911 [-]	1.09E-06	**2.24**
ape0133	APL_0125	hypothetical protein	COG5633	R			141986..142360	1.38E-06	**2.24**
tehB	APL_1350	tellurite resistance protein TehB-like	COG0500	QR			1548925..1549797 [+]	3.34E-10	**2.23**
ape0999	APL_0920	hypothetical protein					1061681..1062937 [+]	3.60E-08	**2.23**
gntR	APL_0571	HTH-type transcriptional regulator	COG1167	KE		Cytoplasmic	643793..645193 [.]	9.05E-11	**2.22**
ape1777	APL_1660	mannose permease IID component	COG3716	G		CytoplasmicMembrane	1886502..1887329 [-]	3.74E-10	**2.22**
amiB	APL_1136	putative N-acetylmuramoyl-L-alanine amidase AmiB precursor	COG0860	M	3.5.1.28		1312068..1313213 [+]	1.54E-06	**2.21**
ape0813	APL_0753	putative esterase/lipase	COG0596	R	3.1.-.-		863424..864209 [+]	7.70E-08	**2.21**
dnaX	APL_0265	DNA polymerase III subunit gamma/tau	COG2812	L	2.7.7.7		293696..295762 [-]	3.59E-04	**2.21**
groEL	APL_1012	60 kDa chaperonin	COG0459	O		Cytoplasmic	1178033..1179676 [-]	4.05E-05	**2.21**
tehA	APL_1212	tellurite resistance protein TehA	COG1275	P		CytoplasmicMembrane	1394716..1395663 [-]	1.43E-03	**2.20**
rnhB	APL_0129	ribonuclease HII	COG0164	L	3.1.26.4		144477..145070 [-]	6.83E-07	**2.20**
ape1601	APL_1491	hypothetical protein					1706547..1707902 [-]	4.26E-05	**2.19**
lolD	APL_2037	Lipoprotein-releasing system ATP-binding protein lolD	COG1136	V		CytoplasmicMembrane	2264515..2265228 [-]	1.49E-06	**2.18**
ape0089	APL_0086	hypothetical protein					94362..94787 [+]	3.70E-07	**2.18**
ape1850	APL_1726	hypothetical protein				Cytoplasmic	1956260..1956808 [+]	9.97E-03	**2.17**
uupA	APL_0294	ABC transporter ATP-binding protein Uup-1	COG0488	R		CytoplasmicMembrane	325804..327747 [+]	8.12E-12	**2.17**
hyaD	APL_1335	hydrogenase 2 maturation protease	COG0680	C		Cytoplasmic	1532789..1533286 [+]	1.97E-06	**2.15**
ppiB	APL_0914	peptidyl-prolyl cis-trans isomerase B	COG0652	O	5.2.1.8	Cytoplasmic	1055847..1056356 [+]	4.42E-08	**2.15**
rho	APL_0247	transcription termination factor Rho	COG1158	K		Cytoplasmic	267745..269007 [-]	2.26E-10	**2.15**
ape0158	APL_0149	hypothetical protein	COG0694	O		Cytoplasmic	167032..167631 [-]	2.02E-05	**2.15**
ape0276	APL_0263	putative ABC transport system permease	COG0842	V		CytoplasmicMembrane	292023..292784 [-]	1.28E-05	**2.15**
afuA_2	APL_0563	Fe3+ ABC transporter, iron-binding protein	COG1840	P		Periplasmic	631946..632977 [+]	2.31E-05	**2.14**
sohB	APL_0008	putative protease SohB	COG0616	OU	3.4.21.-	Cytoplasmic	8624..9679 [+]	5.81E-06	**2.14**
ape0453	APL_0426	hypothetical protein					483270..483728 [-]	2.75E-03	**2.14**
ape1501	APL_1396	hypothetical protein					1596821..1597336 [+]	3.11E-09	**2.13**
ape0223	APL_0213	hypothetical protein	COG1203	R		CytoplasmicMembrane	231296..234643 [-]	1.37E-09	**2.13**
ape1858	APL_1733	hypothetical protein	COG4902				1969302..1969952 [+]	1.00E-08	**2.12**
pepN	APL_1340	aminopeptidase N	COG0308	E	3.4.11.2	Cytoplasmic	1538998..1541607 [+]	5.71E-09	**2.11**
fabZ	APL_0408	(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase	COG0764	I	4.2.1.-	Cytoplasmic	465147..465611 [-]	3.39E-10	**2.11**
ape1827	APL_1707	hypothetical protein					1939172..1939606 [+]	1.79E-04	**2.09**
ape0257	APL_0246	truncated transferrin-binding protein 1 precursor	COG1629	P		OuterMembrane	264910..267177 [+]	4.66E-09	**2.09**
rpoZ	APL_1826	DNA-directed RNA polymerase omega subunit	COG1758	K	2.7.7.6	Cytoplasmic	2049150..2049425 [-]	2.74E-04	**2.09**
ape0805	APL_0745	putative alkaline phosphatase	COG1785	P			851211..852221 [+]	5.55E-07	**2.07**
engC	APL_0130	putative GTPase EngC	COG1162	R	3.6.1.-	Cytoplasmic	145093..146127 [-]	8.01E-08	**2.05**
serA	APL_1452	D-3-phosphoglycerate dehydrogenase	COG0111	HE	1.1.1.95	Cytoplasmic	1664928..1666157 [-]	1.97E-07	**2.05**
fkpA	APL_1640	putative FKBP-type peptidyl-prolyl cis-trans isomerase	COG0545	O	5.2.1.8	Periplasmic	1864839..1865570 [+]	5.41E-06	**2.04**
ape0720	APL_0668	hypothetical protein	COG2822	P			763097..763909 [+]	5.03E-07	**2.04**
murI	APL_1841	glutamate racemase	COG0796	M	5.1.1.3		2064220..2065017 [+]	1.45E-08	**2.04**
pmbA	APL_0729	antibiotic maturation factor	COG0312	R		Cytoplasmic	837571..838920 [-]	4.48E-10	**2.02**
srmB	APL_2008	ATP-dependent RNA helicase	COG0513	LKJ	2.7.7.-	Cytoplasmic	2232346..2233683 [+]	5.85E-10	**2.01**
infB	APL_0639	translation initiation factor IF-2	COG0532	J		Cytoplasmic	730249..732774 [+]	3.11E-05	**2.00**
ape1328	APL_1231	hypothetical protein				Cytoplasmic	1411876..1412367 [.]	5.16E-05	**2.00**
dsbE	APL_1514	thiol:disulfide interchange protein DsbE	COG0526	OC		Periplasmic	1731002..1731544 [+]	8.43E-06	**2.00**
ape0674	APL_0623	hypothetical protein				Cytoplasmic	710915..711133 [-]	1.16E-03	**1.99**
coaA	APL_1513	pantothenate kinase	COG1072	H	2.7.1.33	Cytoplasmic	1729917..1730867 [+]	2.25E-05	**1.99**

dnaJ	APL_1905	Chaperone protein dnaJ	COG0484	O		Cytoplasmic	2135185..2136327 [-]	2.47E-04	**1.98**
ape0506	APL_0472	hypothetical protein				Cytoplasmic	542837..543121 [-]	9.42E-06	**1.98**
gshA	APL_1408	glutathione biosynthesis bifunctional protein GshAB	COG2918	H	6.3.2.2		1609024..1611297 [+]	2.74E-05	**1.97**
lpsA	APL_1118	lipooligosaccharide biosynthesis protein LpsA 3'-end	COG3306	M			1295110..1295580 [-]	3.84E-08	**1.97**
lolE	APL_2036	lipoprotein-releasing system transmembrane protein	COG4591	M		CytoplasmicMembrane	2263265..2264515 [-]	1.43E-06	**1.96**
ape1308	APL_1211	hypothetical protein					1393801..1394637 [+]	5.59E-09	**1.96**
groES	APL_1013	10 kDa chaperonin	COG0234	O		Cytoplasmic	1179740..1180030 [-]	1.29E-03	**1.96**
cpxB	APL_1584	capsule polysaccharide export inner-membrane protein	COG1682	GM		CytoplasmicMembrane	1811593..1812390 [+]	6.34E-06	**1.96**
ape1017	APL_0937	hypothetical protein				Cytoplasmic	1079048..1079590 [-]	3.32E-05	**1.96**
ilvC	APL_1853	Ketol-acid reductoisomerase	COG0059	EH	1.1.1.86		2084660..2086141 [-]	7.98E-08	**1.95**
ape1381	APL_1281	hypothetical protein	COG2166	R			1472393..1472785 [+]	4.65E-05	**1.95**
ompP4	APL_0389	lipoprotein E precursor	COG2503	R			442506..443321 [-]	4.89E-06	**1.94**
ape1041	APL_0959	hemagglutinin/hemolysin-like protein	COG3210	U		OuterMembrane	1104021..1111811 [-]	6.92E-13	**1.94**
ape0840	APL_0780	hypothetical protein	COG1380	R		CytoplasmicMembrane	896661..897020 [-]	1.74E-05	**1.94**
tyrA	APL_0184	T-protein	COG0287	E	5.4.99.5 1.3.1.12	Cytoplasmic	203301..204422 [-]	1.83E-05	**1.93**
smpA	APL_0428	small protein A	COG2913	J			485468..485812 [-]	3.07E-06	**1.93**
ape1984	APL_1855	hypothetical protein	COG1305	E			2087796..2088896 [+]	1.52E-04	**1.93**
ape0907	APL_0838	hypothetical ABC transporter ATP-binding protein	COG1131	V		CytoplasmicMembrane	970713..973454 [+]	1.85E-06	**1.93**
ape0909	APL_0840	predicted outer membrane protein	COG1538	MU		OuterMembrane	974597..975991 [+]	2.56E-05	**1.92**
ape0072	APL_0070	hypothetical protein					83031..83663 [-]	6.48E-09	**1.92**
hisF	APL_2027	Imidazole glycerol phosphate synthase subunit hisF	COG0107	E	4.1.3.-	Cytoplasmic	2254771..2255403 [+]	1.58E-07	**1.91**
ape1186	APL_1096	hypothetical protein	COG3525	G		Periplasmic	1267582..1268715 [+]	3.17E-04	**1.91**
ape2091	APL_1957	Lipoprotein_5 domain containing protein					2185963..2187207 [-]	3.87E-06	**1.91**
argS	APL_0039	arginyl-tRNA synthetase	COG0018	J		Cytoplasmic	42435..44081 [+]	1.71E-04	**1.89**
ape1999	APL_1870	hypothetical protein	COG3087	D		CytoplasmicMembrane	2099777..2100700 [-]	1.33E-06	**1.89**
recX	APL_1142	regulatory protein RecX	COG2137	R			1318240..1318695 [-]	3.70E-05	**1.89**
ape0214	APL_0204	putative threonine efflux protein	COG1280	E		CytoplasmicMembrane	222804..223430 [+]	9.20E-05	**1.89**
ap2140	APL_2003	Hypothetical protein	COG0561	R			2228336..2229145 [-]	5.68E-06	**1.89**
ape1057	APL_0974	hypothetical protein					1127670..1128104 [+]	1.93E-04	**1.88**
secA	APL_0239	preprotein translocase secA subunit	COG0653	U		Cytoplasmic	255285..258002 [+]	1.33E-03	**1.88**
ape0863	APL_0799	hypothetical protein				Cytoplasmic	921300..921911 [+]	2.95E-04	**1.87**
hofQ	APL_0200	type II secretory pathway, component HofQ	COG4796	U		OuterMembrane	219442..220740 [+]	8.06E-06	**1.87**
ape1231	APL_1140	hypothetical protein	COG0009	J		Cytoplasmic	1316862..1317416 [-]	4.48E-04	**1.86**
pflB	APL_1036	formate acetyltransferase	COG1882	C	2.3.1.54	Cytoplasmic	1202932..1205244 [-]	6.11E-07	**1.86**
recO	APL_0545	DNA repair protein RecO	COG1381	L			615674..616396 [+]	2.74E-08	**1.86**
ksgA	APL_0399	dimethyladenosine transferase	COG0030	J	2.1.1.-	Cytoplasmic	453463..454332 [-]	4.05E-03	**1.86**
hybE	APL_1336	hydrogenase-2 operon protein HybE					1533279..1533782 [+]	9.59E-08	**1.85**
ape1188	APL_1098	putative 6-pyruvoyl tetrahydrobiopterin synthase	COG0720	H	4.2.3.12	Cytoplasmic	1269524..1269946 [+]	1.09E-06	**1.85**
ape0224	APL_0214	hypothetical protein	COG1518	L		CytoplasmicMembrane	234640..235596 [-]	2.57E-05	**1.85**
ape0505	APL_0471	hypothetical protein					542535..542828 [-]	3.22E-05	**1.84**
ape1919	APL_1791	putative periplasmic iron/siderophore binding protein	COG0614	P			2014341..2015456 [+]	4.68E-03	**1.84**
ape1625	APL_1515	hypothetical protein	COG3088	O			1731549..1732016 [+]	1.21E-05	**1.83**
trkA	APL_1559	Trk system potassium uptake protein TrkA	COG0569	P			1782709..1784085 [-]	2.95E-04	**1.82**
ape1228	APL_1138	hypothetical protein				Cytoplasmic	1314969..1315220 [-]	3.53E-06	**1.82**
cysE	APL_1511	serine acetyltransferase	COG1045	E	2.3.1.30	Cytoplasmic	1726968..1727783 [+]	7.19E-06	**1.81**
ape2166	APL_2029	Hypothetical protein	COG1738			CytoplasmicMembrane	2256297..2256962 [+]	4.57E-05	**1.80**
ribF	APL_0045	riboflavin biosynthesis protein	COG0196	H	2.7.1.26 2.7.7.2	Cytoplasmic	51401..52363 [-]	5.32E-05	**1.79**
fhuC	APL_2013	Ferrichrome transport ATP-binding protein fhuC	COG1120	PH	3.6.3.34	Cytoplasmic	2238869..2239549 [+]	1.39E-04	**1.79**
pepO	APL_1913	neutral endopeptidase	COG3590	O	3.4.24.-	Cytoplasmic	2146964..2148991 [+]	8.60E-07	**1.79**

ispA	APL_0807	geranyltranstransferase	COG0142	H	2.5.1.10	Cytoplasmic	929306..930199 [+]	1.50E-04	**1.79**
moeB	APL_0311	molybdopterin biosynthesis protein MoeB	COG0476	H		Cytoplasmic	345389..346135 [+]	8.11E-07	**1.78**
folK	APL_0177	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase	COG0801	H	2.7.6.3		195781..196251 [+]	2.27E-03	**1.78**
slmA	APL_1967	HTH-type protein slmA	COG1309	K			2195478..2196086 [-]	1.79E-05	**1.78**
ape0752	APL_0697	putative HTH-type transcriptional regulator	COG0583	K		Cytoplasmic	798742..799638 [+]	1.15E-09	**1.78**
ape0271	APL_0258	hypothetical protein				Cytoplasmic	288590..288886 [-]	1.21E-05	**1.78**
ape0247	APL_0236	putative lipoprotein	COG3015	MP			254045..254419 [-]	1.90E-04	**1.77**
ape1161	APL_1072	Maf-like protein	COG0424	D		Cytoplasmic	1240429..1241019 [-]	8.08E-06	**1.77**
ape0841	APL_0781	putative ATP-dependent helicase	COG1199	KL	3.6.1.-		897087..899009 [-]	2.77E-06	**1.77**
ape0362	APL_0342	hypothetical protein					378173..378733 [-]	4.96E-05	**1.76**
phoB	APL_1257	phosphate regulon transcriptional regulatory protein PhoB	COG0745	TK		Cytoplasmic	1446864..1447505 [-]	7.68E-07	**1.76**
fdhE	APL_0896	formate dehydrogenase accessory protein	COG3058	O			1038255..1039172 [+]	2.68E-04	**1.76**
ape0721	APL_0669	putative iron dependent peroxidase	COG2837	P			763924..765114 [+]	7.61E-04	**1.76**
hybG	APL_1337	hydrogenase-2 operon protein HybG	COG0298	O		Cytoplasmic	1533810..1534088 [+]	4.14E-04	**1.75**
ape0325	APL_0308	hypothetical membrane protein				CytoplasmicMembrane	340397..341443 [+]	1.15E-04	**1.75**
yfeD	APL_0127	putative iron transport system membrane protein	COG1108	P		CytoplasmicMembrane	142799..143629 [-]	3.98E-08	**1.75**
ape1706	APL_1591	hypothetical protein	COG4635	CH			1819377..1819904 [+]	2.09E-04	**1.75**
ape1310	APL_1213	hypothetical protein	COG0637	R		Cytoplasmic	1395762..1396412 [-]	1.35E-03	**1.74**
ape0932	APL_0863	hypothetical protein					1003441..1003605 [+]	5.78E-04	**1.74**
ape0030	APL_0028	ABC transporter permease protein	COG0601	EP		CytoplasmicMembrane	32792..33739 [-]	3.87E-04	**1.73**
ape1884	APL_1758	possible DNA transformation protein	COG3070	K		Cytoplasmic	1995709..1996365 [-]	1.41E-04	**1.72**
znuC	APL_0456	high-affinity zinc uptake system ATP-binding protein ZnuC	COG1121	P		Cytoplasmic	526962..527762 [-]	7.63E-04	**1.72**
ape1998	APL_1869	hypothetical protein				Cytoplasmic	2098821..2099300 [-]	6.07E-07	**1.72**
dxr	APL_0406	1-deoxy-D-xylulose 5-phosphate reductoisomerase	COG0743	I	1.1.1.267	Cytoplasmic	463075..464265 [+]	6.52E-07	**1.71**
dtd	APL_1949	D-tyrosyl-tRNA(Tyr) deacylase	COG1490	J	3.1.-.-		2179788..2180222 [-]	2.59E-06	**1.71**
ape0933	APL_0864	hypothetical protein					1003602..1004054 [+]	1.85E-05	**1.71**
ape0676	APL_0625	hypothetical protein				Cytoplasmic	712379..713152 [-]	3.11E-10	**1.71**
prfA	APL_2044	Peptide chain release factor 1 (RF-1)	COG0216	J		Cytoplasmic	2270835..2271836 [-]	8.06E-05	**1.70**
ape1957	APL_1829	hypothetical protein				Cytoplasmic	2052185..2052472 [-]	3.92E-06	**1.70**
ape1686	APL_1572	hypothetical protein					1799696..1799944 [-]	9.13E-05	**1.69**
recD	APL_0253	exodeoxyribonuclease V alpha chain	COG0507	L	3.1.11.5		276157..278094 [+]	1.77E-07	**1.69**
selB	APL_1561	selenocysteine-specific elongation factor	COG3276	J		Cytoplasmic	1785447..1787282 [-]	6.57E-05	**1.69**
serB	APL_1230	phosphoserine phosphatase	COG0560	E	3.1.3.3		1410957..1411817 [+]	3.03E-06	**1.68**
ape1542	APL_1435	hypothetical protein				CytoplasmicMembrane	1639673..1640185 [-]	1.52E-07	**1.68**
ape1795	APL_1678	putative ferredoxin	COG1145	C			1904636..1905145 [+]	1.89E-02	**1.68**
ape0470	APL_0440	hypothetical protein	COG0121	R		Cytoplasmic	496824..497621 [+]	2.78E-04	**1.68**
ape1332	APL_1234	hypothetical protein				Periplasmic	1418016..1418900 [-]	2.05E-06	**1.67**
ape0908	APL_0839	predicted inner membrane transport permease	COG0842	V		CytoplasmicMembrane	973456..974580 [+]	8.89E-06	**1.67**
trxA	APL_1078	thioredoxin	COG0526	OC		Cytoplasmic	1247188..1247505 [-]	3.16E-04	**1.67**
glnB	APL_1518	nitrogen regulatory protein P-II	COG0347	E		Cytoplasmic	1733706..1734044 [+]	2.84E-06	**1.67**
mscS	APL_1880	Small-conductance mechanosensitive channel	COG0668	M		CytoplasmicMembrane	2109487..2110398 [+]	3.53E-09	**1.67**
ape0772	APL_0715	ABC transport system permease protein	COG4606	P		CytoplasmicMembrane	825251..826198 [+]	6.56E-05	**1.66**
rumA	APL_1112	23S rRNA (uracil-5-)-methyltransferase RumB	COG2265	J	2.1.1.-		1289023..1290198 [+]	2.27E-06	**1.66**
lspA	APL_1519	lipoprotein signal peptidase	COG0597	MU	3.4.23.36	CytoplasmicMembrane	1734123..1734605 [+]	2.06E-05	**1.66**
ape1824	APL_1705	FKBP-type peptidyl-prolyl cis-trans isomerase	COG0545	O	5.2.1.8	OuterMembrane	1937132..1937761 [-]	3.35E-03	**1.65**
ape0208	APL_0198	hypothetical protein				Cytoplasmic	218506..219033 [+]	1.41E-02	**1.65**
tdk	APL_0622	thymidine kinase	COG1435	F	2.7.1.21	Cytoplasmic	710274..710855 [-]	1.49E-02	**1.64**
ape0245	APL_0234	putative lipoprotein	COG3056	M			252622..253212 [-]	2.00E-04	**1.64**

accD	APL_0631	acetyl-coenzyme A carboxylase carboxyl transferase subunit beta	COG0777	I	6.4.1.2		718567..719460 [+]	1.08E-04	**1.64**
cdd	APL_1343	cytidine deaminase	COG0295	F	3.5.4.5		1542869..1543762 [+]	9.71E-07	**1.64**
ape0978	APL_0905	hypothetical protein					1047346..1047684 [-]	8.00E-05	**1.63**
ape0152	APL_0144	predicted ATP-dependent endonuclease of the OLD family	COG3593	L		Cytoplasmic	159936..161495 [-]	2.21E-09	**1.63**
ape1776	APL_1659	hypothetical protein					1886037..1886414 [-]	1.54E-05	**1.63**
ape1863	APL_1738	lactoylglutathione lyase and related lyases	COG0346	E	4.4.1.5	Cytoplasmic	1973932..1974324 [-]	7.05E-06	**1.63**
yfeC	APL_0128	putative iron transport system membrane protein	COG1108	P		CytoplasmicMembrane	143622..144476 [-]	3.43E-09	**1.63**
D15	APL_0411	protective surface antigen D15 precursor	COG4775	M		OuterMembrane	467627..470008 [-]	3.12E-06	**1.63**
trpCF	APL_0859	tryptophan biosynthesis protein trpCF [Includes: Indole-3-glycerol phosphate synthase; N-(5'-phospho-ribosyl)anthranilate isomerase]	COG0134	E	4.1.1.48 5.3.1.24		1000124..1001533 [-]	6.54E-07	**1.63**
macB	APL_0626	putative macrolide-specific ABC-type efflux carrier	COG0577	V		CytoplasmicMembrane	713244..715166 [-]	4.02E-06	**1.62**
thiP	APL_1321	thiamine transport system permease protein ThiP	COG1178	P		CytoplasmicMembrane	1513393..1515018 [-]	1.44E-04	**1.62**
ape1369	APL_1269	putative ribonuclease	COG3719	J			1458764..1459531 [+]	1.27E-03	**1.62**
ape0771	APL_0714	ABC transport system periplasmic protein	COG4607	P		Periplasmic	824256..825164 [+]	5.23E-06	**1.62**
xylA	APL_1908	Xylose isomerase	COG2115	G	5.3.1.5	Cytoplasmic	2140373..2141692 [-]	1.81E-03	**1.62**
pepQ	APL_0091	Xaa-Pro dipeptidase	COG0006	E	3.4.13.9		97683..99014 [-]	8.92E-06	**1.62**
ape0457	APL_0429	nucleoid-associated protein NdpA	COG3081	R		Cytoplasmic	485883..486884 [-]	1.22E-08	**1.62**
ape1815	APL_1697	hypothetical protein				OuterMembrane	1928704..1929798 [-]	3.01E-04	**1.61**
ape0906	APL_0837	hypothetical protein	COG0845	M		OuterMembrane	969648..970709 [+]	8.55E-03	**1.61**
ape0029	APL_0027	nickel transport system permease protein	COG1173	EP		CytoplasmicMembrane	32004..32789 [-]	1.10E-04	**1.61**
hslO	APL_1315	heat shock-like protein 33	COG1281	O		Cytoplasmic	1505774..1506664 [+]	1.09E-03	**1.60**
moaE	APL_0693	molybdopterin-converting factor subunit 2	COG0314	H			793098..793553 [+]	4.55E-05	**1.60**
alaS	APL_0654	alanyl-tRNA synthetase	COG0013	J	6.1.1.7	Cytoplasmic	747203..749827 [-]	4.18E-06	**1.60**
aroF	APL_0185	phospho-2-dehydro-3-deoxyheptonate aldolase, Tyr-sensitive	COG0722	E	2.5.1.54		204535..205596 [-]	2.97E-05	**1.59**
ape0846	APL_0784	hypothetical protein					903161..903979 [-]	6.70E-08	**1.59**
ptsH	APL_1322	PTS system phosphocarrier protein HPr	COG1925	G		Cytoplasmic	1515255..1515512 [+]	8.03E-03	**1.59**
truC	APL_0862	tRNA pseudouridine synthase C	COG0564	J	5.4.99.12	Cytoplasmic	1002696..1003439 [+]	1.51E-06	**1.59**
proQ	APL_0119	proQ-like protein	COG3109	T			135550..136098 [+]	7.13E-04	**1.59**
ape1679	APL_1565	putative gluconolactonase	COG3386	G	3.1.1.17		1791594..1792037 [-]	1.94E-02	**1.59**
ape1870	APL_1745	hypothetical protein	COG3636	K		Cytoplasmic	1982555..1982863 [-]	7.73E-05	**1.59**
psiE	APL_0069	phosphate-starvation-inducible protein PsiE				CytoplasmicMembrane	82543..82953 [-]	8.75E-06	**1.58**
ape0209	APL_0199	hypothetical protein					219026..219430 [+]	1.06E-02	**1.57**
ape0124	APL_0116	133224..133808 [+]					133224..133808 [+]	2.33E-10	**1.57**
ape1775	APL_1658	hypothetical protein					1885558..1886034 [-]	5.43E-05	**1.57**
ape0256	APL_0245	transferrin binding protein-like solute binding protein				OuterMembrane	263186..264895 [+]	4.07E-06	**1.57**
ape0543	APL_0501	possible DNA methylase					570693..571217 [+]	4.92E-03	**1.56**
yecO	APL_0754	S-adenosyl-L-methionine-dependent methyltransferase	COG0500	QR		Cytoplasmic	864217..864942 [+]	8.83E-05	**1.56**
fic	APL_0860	filamentation induced by cAMP protein Fic-like				Cytoplasmic	1001711..1002376 [+]	4.64E-04	**1.56**
rec2	APL_0766	recombination protein 2	COG0658	R		CytoplasmicMembrane	875330..877624 [-]	2.68E-05	**1.56**
mtn	APL_1637	MTA/SAH nucleosidase	COG0775	F	3.2.2.16 3.2.2.9		1863055..1863753 [-]	9.04E-03	**1.56**
ape2034	APL_1903	hypothetical protein	COG2819	R			2133195..2134019 [+]	1.71E-08	**1.55**
rnb	APL_0757	exoribonuclease 2	COG4776	K	3.1.13.1	Cytoplasmic	866787..868763 [+]	4.08E-03	**1.55**
dcd	APL_0835	deoxycytidine triphosphate deaminase	COG0717	F	3.5.4.13	Cytoplasmic	968300..968884 [+]	8.12E-03	**1.55**
ape2052	APL_1920	site-specific recombinase	COG4389	L		CytoplasmicMembrane	2153314..2155281 [+]	7.45E-06	**1.55**
ape1648	APL_1536	N-acetylmuramic acid 6-phosphate etherase	COG2103	R			1750596..1751510 [+]	8.55E-05	**1.55**
murC	APL_0019	UDP-N-acetylmuramate--L-alanine ligase	COG0773	M	6.3.2.8	Cytoplasmic	21880..23307 [+]	1.37E-04	**1.55**
ape0028	APL_0026	ABC transporter ATP-binding protein	COG1123	R		CytoplasmicMembrane	30601..32004 [-]	6.12E-05	**1.54**
moeA	APL_0310	molybdopterin biosynthesis protein MoeA	COG0303	H			344138..345379 [+]	1.99E-06	**1.54**
xseA	APL_0817	putative exodeoxyribonuclease VII large subunit	COG1570	L	3.1.11.6		943691..945151 [+]	2.47E-05	**1.53**

aroE	APL_1139	shikimate dehydrogenase	COG0169	E	1.1.1.25	Cytoplasmic	1316034..1316855 [-]	1.44E-07	**1.53**
recG	APL_1827	ATP-dependent DNA helicase recG	COG1200	LK	3.6.1.-		2049552..2051633 [-]	5.60E-07	**1.52**
ape1825	APL_1706	hypothetical protein				CytoplasmicMembrane	1938143..1938826 [+]	2.92E-05	**1.52**
ape0960	APL_0889	hypothetical protein					1029866..1031281 [+]	2.87E-05	**1.52**
kdkA	APL_0904	3-deoxy-D-manno-octulosonic acid kinase	COG0515	RTKL	2.7.1.-	Cytoplasmic	1046562..1047266 [+]	3.16E-08	**1.52**
dcuB2	APL_1316	anaerobic C4-dicarboxylate transporter DcuB	COG2704	R		CytoplasmicMembrane	1506732..1508066 [-]	1.13E-04	**1.51**
djlA	APL_0306	DnaJ-like protein DjlA	COG1076	O		Cytoplasmic	338438..339298 [+]	1.67E-05	**1.51**
ape1857	APL_1732	hypothetical protein	COG3059			CytoplasmicMembrane	1968640..1969056 [-]	5.58E-05	**1.51**
scrR	APL_2032	Sucrose operon repressor (Scr operon regulatory protein)	COG1609	K		Cytoplasmic	2258469..2259488 [-]	1.19E-04	**1.51**
flp1	APL_0559	fimbrial protein Flp precursor	COG3847	U			627628..627858 [-]	4.55E-04	**1.50**
ape0283	APL_0270	hypothetical protein					298665..299156 [+]	4.72E-04	**1.50**
apxIB	APL_1443	toxin RTX-I translocation ATP-binding protein	COG2274	V		CytoplasmicMembrane	1650723..1652846 [-]	6.76E-03	**1.50**
ruvB	APL_0284	holliday junction ATP-dependent DNA helicase RuvB	COG2255	L		Cytoplasmic	313779..314663 [+]	2.31E-07	**1.50**
ape0444	APL_0418	hypothetical protein	COG1534	J			475651..476052 [-]	7.22E-03	**1.50**
ape1307	APL_1210	hypothetical protein	COG1670	J			1393091..1393801 [+]	8.83E-03	**1.50**

G 4 Genes downregulated by HlyX as obtained by microarray analysis

gene	locus_tag	gene product	COG #	COG	EC #	psorb_loc	location	T-test	ratio
sodA	APL_0251	manganese superoxide dismutase	COG0605	P	1.15.1.1	Cytoplasmic	274183..274824 [+]	9.47E-07	**-5.37**
ape0688	APL_0637	hypothetical protein				Cytoplasmic	728268..728729 [+]	1.27E-05	**-5.32**
ptnD	APL_1393	mannose permease IID component	COG3716	G		CytoplasmicMembrane	1594164..1595000 [+]	7.08E-13	**-5.19**
ape1496	APL_1391	PTS system mannose-specific EIIAB component [Includes: Mannose-specific phosphotransferase enzyme IIA component; Mannose-specific phosphotransferase enzyme IIB component]	COG3444	G	2.7.1.69	Cytoplasmic	1592366..1593337 [+]	3.32E-06	**-5.04**
glmS	APL_1631	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]	COG0449	M	2.6.1.16	Cytoplasmic	1855480..1857312 [-]	1.21E-05	**-4.89**
ptnC	APL_1392	mannose permease IIC component	COG3715	G		CytoplasmicMembrane	1593350..1594150 [+]	2.23E-05	**-4.45**
pryE	APL_0318	orotate phosphoribosyltransferase	COG0461	F	2.4.2.10		354902..355543 [+]	5.05E-06	**-4.33**
nrdA	APL_0992	ribonucleoside-diphosphate reductase alpha subunit	COG0209	F	1.17.4.1	OuterMembrane	1145469..1147739 [+]	2.40E-05	**-4.16**
ape1747	APL_1632	predicted transcriptional regulator of sugar metabolism	COG1349	KG		Cytoplasmic	1857436..1858194 [-]	2.35E-06	**-4.12**
ape2067	APL_1934	hypothetical protein	COG0700			CytoplasmicMembrane	2169338..2169802 [-]	1.20E-05	**-3.98**
apfC	APL_0878	fimbrial biogenesis protein	COG1459	NU		CytoplasmicMembrane	1018037..1019239 [-]	2.74E-05	**-3.86**
ape1478	APL_1373	hypothetical protein	COG0593	L		Cytoplasmic	1569626..1570336 [-]	5.07E-07	**-3.77**
ape0338	APL_0319	hypothetical protein	COG0620	E		Cytoplasmic	355682..356770 [+]	1.75E-06	**-3.59**
dhaL	APL_0082	PTS-dependent dihydroxyacetone kinase, ADP-binding subunit DhaL	COG2376	G	2.7.1.-		90557..91183 [-]	6.06E-10	**-3.59**
dhaK	APL_0083	PTS-dependent dihydroxyacetone kinase, dihydroxyacetone-binding subunit DhaK	COG2376	G	2.7.1.-		91185..92255 [-]	5.95E-06	**-3.48**
ihfB	APL_0739	integration host factor beta-subunit	COG0776	L			844554..844835 [-]	9.89E-06	**-3.43**
ape2068	APL_1935	hypothetical protein				CytoplasmicMembrane	2169799..2170224 [-]	1.68E-05	**-3.34**
fruK	APL_0344	1-phosphofructokinase	COG1105	G	2.7.1.56	Cytoplasmic	380652..381599 [-]	1.02E-08	**-3.33**
ape2066	APL_1933	acetylornithine deacetylase/succinyl-diaminopimelate desuccinylase and related deacylases	COG0624	E		Cytoplasmic	2168701..2169324 [-]	4.37E-09	**-3.32**
ape0172	APL_0163	anaerobic ribonucleoside triphosphate reductase	COG1328	F			180719..182578 [-]	3.20E-05	**-3.27**
metC	APL_0320	cystathionine beta-lyase	COG0626	E	4.4.1.8		356847..358037 [-]	1.07E-05	**-3.22**
ulaG	APL_1701	L-ascorbate-6-phosphate lactonase UlaG-like	COG2220	R		Cytoplasmic	1933388..1934479 [+]	1.50E-06	**-3.17**
pyrD	APL_0774	dihydroorotate dehydrogenase	COG0167	F	1.3.3.1	Cytoplasmic	890441..891448 [-]	1.49E-08	**-3.16**
ape0917	APL_0848	putative ABC transporter periplasmic binding protein	COG0747	E		CytoplasmicMembrane	983413..984987 [+]	3.52E-05	**-3.09**
afuA	APL_1446	ABC-type Fe3+ transport system, periplasmic component	COG1840	P		Periplasmic	1656814..1657854 [+]	7.34E-06	**-3.08**
ansB	APL_0135	probable L-asparaginase periplasmic precursor	COG0252	EJ	3.5.1.1	Periplasmic	149916..150965 [-]	3.68E-10	**-3.07**
ape1770	APL_1653	hypothetical protein	COG3312	C		CytoplasmicMembrane	1879898..1880281 [-]	2.16E-07	**-3.07**
aceE	APL_0773	pyruvate dehydrogenase E1 component	COG2609	C	1.2.4.1		887432..890086 [-]	7.10E-08	**-3.05**
fbp	APL_1450	fructose-1,6-bisphosphatase	COG0158	G	3.1.3.11		1662693..1663697 [+]	1.72E-13	**-3.04**
ape0574	APL_0528	putative transporter	COG1115	E		CytoplasmicMembrane	601844..603292 [+]	1.22E-06	**-3.01**
afuB	APL_1447	Ferric transport system permease protein fbpB	COG1178	P		CytoplasmicMembrane	1657959..1660022 [+]	1.72E-06	**-3.01**
fruB	APL_0345	multiphosphoryl transfer protein (Includes: Phosphocarrier protein HPr; PTS system fructose-specific EIIA component)	COG4668	G	2.7.1.69	Cytoplasmic	381614..383122 [-]	1.38E-07	**-2.97**
lpdA	APL_0771	dihydrolipoyl dehydrogenase	COG1249	C	1.8.1.4	Cytoplasmic	883954..885378 [-]	2.22E-08	**-2.96**
ribD	APL_0382	riboflavin biosynthesis protein (Includes: Diaminohydroxyphosphoribosylaminopyrimidine deaminase; 5-amino-6-(5-phosphoribosylamino)uracil reductase)	COG1985	H	1.1.1.193 3.5.4.26	Cytoplasmic	435609..436841 [+]	2.69E-06	**-2.96**
ape0778	APL_0719	putative phosphate permease	COG0306	P		CytoplasmicMembrane	828778..830040 [+]	2.51E-06	**-2.94**
ape0777	APL_0718	hypothetical protein	COG1392	P		Cytoplasmic	828088..828768 [+]	3.95E-07	**-2.92**
fruA	APL_0343	PTS system fructose-specific EIIBC component (Includes: Fructose-specific phosphotransferase enzyme IIB component; Fructose permease IIC component)	COG1299	G		CytoplasmicMembrane	378909..380570 [-]	4.52E-06	**-2.92**
putA	APL_0106	bifunctional protein PutA [Includes: Proline dehydrogenase; Delta-1-pyrroline-5-carboxylate dehydrogenase]	COG4230	C	1.5.1.12 1.5.99.8	Cytoplasmic	121566..125171 [-]	1.29E-09	**-2.86**
rpsA	APL_0740	30S ribosomal protein S1	COG0539	J		Cytoplasmic	844909..846573 [-]	4.73E-04	**-2.85**
ape1042	APL_0960	putative hemolysin activation/secretion protein	COG2831	U		OuterMembrane	1111859..1112911 [-]	1.30E-06	**-2.84**

afuC	APL_1448	ferric ABC transporter ATP-binding protein	COG3842	E	3.6.3.30		1660069..1661115 [+]	3.18E-07	**-2.83**
rpe	APL_1820	ribulose-phosphate 3-epimerase	COG0036	G	5.1.3.1	Cytoplasmic	2042459..2043133 [-]	4.01E-05	**-2.81**
ape0940	APL_0870	putative C4-dicarboxylate transporter	COG3069	C		CytoplasmicMembrane	1009886..1011178 [-]	1.66E-05	**-2.79**
atpB	APL_1652	ATP synthase A chain	COG0356	C	3.6.3.14	CytoplasmicMembrane	1879085..1879873 [-]	1.01E-06	**-2.79**
ape0173	APL_0164	hypothetical protein	COG0602	O			182583..183050 [-]	5.35E-06	**-2.79**
tktA	APL_0983	transketolase 2	COG0021	G	2.2.1.1	Cytoplasmic	1136203..1138209 [+]	3.08E-05	**-2.76**
rplS	APL_1789	50S ribosomal protein L19	COG0335	J		Cytoplasmic	2012412..2012762 [+]	1.09E-10	**-2.76**
tpiA	APL_1925	Triosephosphate isomerase	COG0149	G	5.3.1.1		2161944..2162714 [-]	1.69E-06	**-2.75**
gpt	APL_0255	xanthine phosphoribosyltransferase	COG0503	F	2.4.2.22	Cytoplasmic	285869..286342 [+]	1.21E-09	**-2.75**
ape1523	APL_1416	hypothetical protein					1619045..1619386 [+]	2.73E-05	**-2.75**
pqiA	APL_1854	hypothetical protein				CytoplasmicMembrane	2086378..2087619 [+]	1.10E-07	**-2.72**
ape0573	APL_0527	predicted ABC-type transport system, permease component	COG2215	R		CytoplasmicMembrane	600881..601744 [+]	2.61E-09	**-2.72**
gmk	APL_0256	guanylate kinase	COG0194	F	2.7.4.8	Cytoplasmic	286426..287046 [+]	4.52E-06	**-2.72**
trmD	APL_1788	tRNA (guanine-N(1)-)-methyltransferase	COG0336	J	2.1.1.31	Cytoplasmic	2011631..2012386 [+]	4.14E-07	**-2.71**
atpF	APL_1650	ATP synthase B chain	COG0711	C	3.6.3.14		1878182..1878652 [-]	2.29E-06	**-2.68**
ape0233	APL_0222	putative lipoprotein					244701..245360 [+]	1.70E-05	**-2.67**
metR	APL_1158	HTH-type transcriptional regulator MetR	COG0583	K		Cytoplasmic	1334889..1335818 [+]	1.08E-06	**-2.66**
betT	APL_1209	putative choline-glycine betaine transporter	COG1292	M		CytoplasmicMembrane	1390767..1392794 [	7.25E-07	**-2.65**
apfD	APL_0877	fimbrial leader peptidase	COG1989	NOU		CytoplasmicMembrane	1017372..1018010 [-]	4.56E-05	**-2.65**
atpE	APL_1651	ATP synthase subunit C	COG0636	C	3.6.3.14	CytoplasmicMembrane	1878712..1878966 [-]	4.48E-13	**-2.65**
ape0231	APL_0220	putative lipoprotein	COG1462	M			243658..244332 [+]	3.32E-07	**-2.61**
atpH	APL_1649	ATP synthase delta chain	COG0712	C	3.6.3.14	Cytoplasmic	1877635..1878168 [-]	4.52E-07	**-2.60**
dhaM	APL_0081	PTS-dependent dihydroxyacetone kinase, phosphotransferase subunit DhaM					90141..90548 [-]	3.28E-06	**-2.60**
ape1194	APL_1104	hypothetical protein	COG0795	R		CytoplasmicMembrane	1276024..1277106 [+]	7.19E-11	**-2.56**
arcD	APL_1083	putative arginine/ornithine antiporter	COG0531	E		CytoplasmicMembrane	1252962..1254368 [+]	7.30E-10	**-2.56**
gidB	APL_1654	methyltransferase GidB	COG0357	M	2.1.-.-		1880642..1881259 [-]	2.10E-07	**-2.56**
ape1320	APL_1223	putative inner membrane protein	COG2252	R		CytoplasmicMembrane	1405767..1407062 [+]	8.21E-08	**-2.55**
rimM	APL_1787	16S rRNA-processing protein rimM	COG0806	J		Cytoplasmic	2011046..2011573 [+]	4.96E-05	**-2.55**
deaD	APL_0575	cold-shock DEAD box protein A-like	COG0513	LKJ		Cytoplasmic	647423..649282 [-]	5.21E-07	**-2.55**
aceF	APL_0772	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)	COG0508	C	2.3.1.12	Cytoplasmic	885474..887372 [-]	2.86E-06	**-2.53**
purD	APL_1172	phosphoribosylamine--glycine ligase	COG0151	F	6.3.4.13		1347552..1348838 [+]	5.16E-06	**-2.52**
ape0795	APL_0735	hypothetical protein	COG0023	J			841891..842202 [-]	2.27E-08	**-2.52**
ape1075	APL_0991	hypothetical protein	COG1408	R		CytoplasmicMembrane	1144177..1145256 [+]	1.06E-14	**-2.49**
aspA	APL_1091	aspartate ammonia-lyase	COG1027	E	4.3.1.1	Cytoplasmic	1261854..1263278 [-]	3.02E-05	**-2.47**
ape0827	APL_0767	hypothetical symporter	COG3633	E		CytoplasmicMembrane	877724..878938 [-]	4.23E-04	**-2.46**
lpxK	APL_1278	tetraacyldisaccharide 4'-kinase	COG1663	M	2.7.1.130		1469348..1470328 [-]	2.88E-08	**-2.46**
pntB	APL_0841	NAD(P) transhydrogenase subunit beta	COG1282	C	1.6.1.2	CytoplasmicMembrane	976069..977517 [-]	6.06E-05	**-2.45**
ape1499	APL_1394	hypothetical protein				CytoplasmicMembrane	1595099..1595557 [+]	2.71E-08	**-2.44**
ape2065	APL_1932	putative carboxypeptidase	COG0624	E		Cytoplasmic	2168196..2168642 [-]	1.35E-06	**-2.44**
mreB	APL_0435	rod shape-determining protein MreB	COG1077	D		Cytoplasmic	491720..492772 [+]	9.64E-07	**-2.43**
adhC	APL_1208	putative alcohol dehydrogenase class 3	COG1062	C	1.1.1.1 1.1.1.284	Cytoplasmic	1389494..1390612 [-]	1.35E-08	**-2.41**
priB	APL_1170	primosomal replication protein n	COG2965	L			1346539..1346913 [-]	8.27E-10	**-2.39**
ape0798	APL_0738	hypothetical protein					844195..844485 [-]	3.36E-06	**-2.39**
mreC	APL_0436	rod shape-determining protein MreC	COG1792	M			492889..493938 [+]	5.07E-07	**-2.38**
ape0636	APL_0585	hypothetical protein	COG1309	K		Cytoplasmic	665452..666030 [+]	5.89E-06	**-2.38**
ape0868	APL_0804	hypothetical protein	COG1368	M		CytoplasmicMembrane	926126..928102 [-]	1.83E-05	**-2.36**
plsX	APL_1385	fatty acid/phospholipid synthesis protein PlsX	COG0416	I			1585942..1586976 [-]	1.96E-08	**-2.36**
ape1193	APL_1103	hypothetical protein	COG0795	R		CytoplasmicMembrane	1274992..1276023 [+]	1.71E-06	**-2.35**

ape0670	APL_0619	hypothetical protein	COG2321	R			706979..707842 [+]	4.72E-07	**-2.34**
oadB	APL_1377	oxaloacetate decarboxylase beta chain	COG1883	C	4.1.1.3	CytoplasmicMembrane	1579435..1580739 [+]	4.07E-08	**-2.33**
surE	APL_1927	5'-nucleotidase surE	COG0496	R	3.1.3.5	Cytoplasmic	2164002..2164766 [+]	8.17E-07	**-2.32**
pepE	APL_0871	peptidase E	COG3340	E	3.4.13.21	Cytoplasmic	1011189..1011893 [-]	5.23E-05	**-2.32**
ape1221	APL_1131	hypothetical protein	COG3307	M		CytoplasmicMembrane	1307881..1309230 [+]	1.02E-08	**-2.31**
atpC	APL_1645	ATP synthase epsilon chain	COG0355	C	3.6.3.14	Cytoplasmic	1873346..1873765 [-]	5.69E-05	**-2.30**
tldD	APL_1540	TldD-like protein	COG0312	R		Cytoplasmic	1753394..1754854 [-]	1.95E-05	**-2.30**
ape1218	APL_112	membrane protein	COG0628	R		CytoplasmicMembrane	1304670..1305722 [-]	3.22E-04	**-2.30**
atpG	APL_1647	ATP synthase gamma chain	COG0224	C	3.6.3.14		1875192..1876058 [-]	4.36E-06	**-2.29**
ape0331	APL_0313	deoxyguanosinetriphosphate triphosphohydrolase-like protein	COG0232	F	3.1.5.1	Cytoplasmic	347915..349240 [+]	5.78E-06	**-2.29**
pnuC	APL_1173	nicotinamide mononucleotide transporter	COG3201	H		CytoplasmicMembrane	1348930..1349622 [-]	4.75E-06	**-2.29**
pntA	APL_0842	NAD(P) transhydrogenase subunit alpha	COG3288	C	1.6.1.2	CytoplasmicMembrane	977528..979066 [-]	2.91E-05	**-2.28**
ape0662	APL_0611	putative lipoprotein	COG0791	M			697885..698409 [+]	1.27E-06	**-2.28**
degS	APL_0742	protease DegS precursor	COG0265	O	3.4.21.-	Periplasmic	847374..848405 [-]	1.72E-05	**-2.28**
acpP	APL_1819	acyl carrier protein	COG0236	IQ		Cytoplasmic	2041962..2042195 [-]	3.59E-09	**-2.27**
ulaA	APL_1700	Predicted ascorbate-specific permease IIC component				CytoplasmicMembrane	1931109..1932890 [-]	9.12E-07	**-2.27**
oadA	APL_1376	oxaloacetate decarboxylase alpha chain	COG5016	C	4.1.1.3		1577610..1579418 [+]	3.97E-08	**-2.26**
galK	APL_0995	galactokinase	COG0153	G	2.7.1.6	Cytoplasmic	1150505..1151659 [+]	5.38E-05	**-2.25**
ape1414	APL_1313	putative ADP compounds hydrolase	COG0494	LR	3.6.1.-	Cytoplasmic	1504539..1505096 [-]	5.21E-09	**-2.25**
ape2061	APL_1929	lipoprotein	COG0739	M			2165362..2165937 [+]	2.01E-06	**-2.25**
smf	APL_1712	Protein smf (DNA-processing chain A)	COG0758	LU		Cytoplasmic	1942973..1944127 [+]	2.31E-08	**-2.25**
nqrC	APL_0152	Na(+)-translocating NADH-quinone reductase subunit C	COG2869	C	1.6.5.-		170675..171448 [+]	2.87E-09	**-2.24**
rpmI	APL_0224	50S ribosomal protein L35	COG0291	J			246366..246563 [+]	2.99E-05	**-2.24**
mreD	APL_0437	rod shape-determining protein MreD	COG2891	M		CytoplasmicMembrane	493938..494426 [+]	1.04E-08	**-2.23**
secG	APL_0743	protein-export membrane protein	COG1314	U		CytoplasmicMembrane	848654..848980 [-]	4.42E-06	**-2.23**
ape0916	APL_0847	oxygen-independent coproporphyrinogen III oxidase-like protein	COG0635	H	1.3.99.22	Cytoplasmic	981993..983150 [-]	1.86E-05	**-2.23**
rpsJ	APL_1759	30S ribosomal protein S10	COG0051	J			1996773..1997084 [+]	7.33E-05	**-2.23**
ape1401	APL_1300	hypothetical protein	COG0477	GEPR		CytoplasmicMembrane	1489613..1490893 [-]	1.08E-05	**-2.22**
ftsJ	APL_0594	ribosomal RNA large subunit methyltransferase J	COG0293	J	2.1.1.-		678772..679398 [+]	3.19E-07	**-2.22**
rplI	APL_1169	50S ribosomal protein L9	COG0359	J		Cytoplasmic	1345870..1346319 [-]	1.35E-10	**-2.21**
abgB	APL_0869	aminobenzoyl-glutamate utilization-like protein	COG1473	R		Cytoplasmic	1008524..1009792 [-]	8.39E-06	**-2.20**
ape1102	APL_1017	hypothetical protein				CytoplasmicMembrane	1183417..1184061 [+]	2.67E-08	**-2.20**
ape0779	APL_0720	hypothetical protein	COG3103	T			830104..830709 [+]	6.09E-10	**-2.20**
kpsF	APL_1576	arabinose-5-phosphate isomerase	COG0794	M		Cytoplasmic	1802526..1803461 [-]	7.74E-08	**-2.18**
atpD	APL_1646	ATP synthase subunit beta	COG0055	C	3.6.3.14	Cytoplasmic	1873805..1875178 [-]	2.18E-10	**-2.17**
rbsB	APL_1672	D-ribose-binding periplasmic protein precursor	COG1879	G		Periplasmic	1898002..1898874 [+]	1.51E-05	**-2.17**
gidA	APL_1655	tRNA uridine 5-carboxymethylaminomethyl modification enzyme GidA	COG0445	D			1881359..1883251 [-]	5.25E-09	**-2.16**
ape0786	APL_0726	carbonic anhydrase	COG0288	P	4.2.1.1	Cytoplasmic	834060..834776 [+]	5.76E-07	**-2.16**
ape1492	APL_1387	hypothetical protein	COG1399	R		Cytoplasmic	1587189..1587716 [-]	1.55E-09	**-2.16**
norM	APL_0369	putative multidrug resistance protein NorM	COG0534	V		CytoplasmicMembrane	414627..416018 [+]	9.23E-06	**-2.15**
cmkA	APL_0741	cytidylate kinase	COG0283	F	2.7.4.14	Cytoplasmic	846670..847344 [-]	8.71E-07	**-2.14**
ape1319	APL_1222	aspartate-semialdehyde dehydrogenase	COG0136	E			1404705..1405706 [+]	5.14E-08	**-2.14**
rplX	APL_1770	50S ribosomal protein L24	COG0198	J		Cytoplasmic	2002361..2002672 [+]	8.94E-09	**-2.13**
sbcB	APL_0673	exodeoxyribonuclease I	COG2925	L	3.1.11.1	Cytoplasmic	767214..768758 [-]	3.70E-07	**-2.13**
ape1410	APL_1309	putative permease	COG0477	GEPR		CytoplasmicMembrane	1499927..1501249 [-]	7.00E-07	**-2.13**
tatC	APL_1987	Sec-independent protein translocase protein tatC	COG0805	U		CytoplasmicMembrane	2214043..2214615 [+]	3.76E-11	**-2.13**
fabI	APL_0755	enoyl-[acyl-carrier-protein] reductase [NADH]	COG0623	I	1.3.1.9	Cytoplasmic	865141..865929 [+]	4.79E-06	**-2.13**
glpT	APL_0377	glycerol-3-phosphate transporter	COG2271	G		CytoplasmicMembrane	428217..429662 [-]	1.50E-08	**-2.12**
pgl	APL_1310	6-phosphogluconolactonase	COG0363	G	3.1.1.31	Cytoplasmic	1501382..1502080 [-]	4.04E-11	**-2.12**

mpl	APL_1449	UDP-N-acetylmuramate:L-alanyl-gamma-D-glutamyl-meso-diaminopimelate ligase	COG0773	M	6.3.2.-		1661206..1662570 [-]	1.22E-09	**-2.12**
hemC	APL_1010	porphobilinogen deaminase	COG0181	H	2.5.1.61	Cytoplasmic	1174046..1174975 [-]	4.00E-05	**-2.12**
ape0459	APL_0431	hypothetical protein				Cytoplasmic	487394..488131 [+]	1.45E-05	**-2.12**
rnfA	APL_0165	electron transport complex protein RnfA	COG4657	C		CytoplasmicMembrane	183432..184013 [+]	3.85E-07	**-2.12**
ape1350	APL_1252	hypothetical protein	COG0471	P		CytoplasmicMembrane	1438796..1440184 [-]	6.61E-05	**-2.11**
dsbB	APL_0321	disulfide bond formation protein B	COG1495	O		CytoplasmicMembrane	358114..358653 [-]	1.61E-04	**-2.11**
galM	APL_0996	aldose 1-epimerase	COG2017	G		Periplasmic	1151717..1152685 [+]	8.43E-06	**-2.11**
tyrP-A	APL_0596	tyrosine-specific transport protein 1	COG0814	E		CytoplasmicMembrane	681662..682876 [+]	7.09E-06	**-2.11**
palA	APL_0304	outer membrane protein precursor PalA	COG2885	M		OuterMembrane	336904..337374 [+]	1.86E-07	**-2.11**
nqrD	APL_0153	Na(+)-translocating NADH-quinone reductase subunit D	COG1347	C	1.6.5.-	CytoplasmicMembrane	171448..172071 [+]	3.56E-07	**-2.10**
dnaE	APL_1105	DNA polymerase III subunit alpha	COG0587	L	2.7.7.7	Cytoplasmic	1277313..1280789 [+]	3.63E-07	**-2.10**
ppx	APL_0708	exopolyphosphatase	COG0248	FP	3.6.1.11	Cytoplasmic	817862..819370 [-]	6.24E-07	**-2.10**
ape1173	APL_1084	hypothetical protein				CytoplasmicMembrane	1254377..1255156 [+]	4.88E-05	**-2.09**
ape1346	APL_1248	AzlC family protein	COG1296	E		CytoplasmicMembrane	APL_1248	6.93E-07	**-2.09**
apxIIA	APL_0956	RTX-II toxin determinant A				Extracellular	1095621..1098488 [-]	4.87E-04	**-2.09**
ape1651	APL_1539	hypothetical protein				Cytoplasmic	1753013..1753273 [-]	1.24E-07	**-2.08**
nqrE	APL_0154	Na(+)-translocating NADH-quinone reductase subunit E	COG2209	C	1.6.5.-	CytoplasmicMembrane	172073..172669 [+]	2.58E-06	**-2.08**
ampD	APL_0316	signalling protein AmpD	COG3023	V		Cytoplasmic	353304..353852 [-]	1.40E-06	**-2.08**
rplO	APL_1778	50S ribosomal protein L15	COG0200	J		Cytoplasmic	2005599..2006033 [+]	3.05E-08	**-2.08**
ape1060	APL_0976	hypothetical protein	COG2056	R		CytoplasmicMembrane	1129435..1130811 [+]	1.91E-06	**-2.08**
hbpA	APL_2010	Heme-binding protein A	COG0747	E		Periplasmic	2234459..2236096 [-]	8.79E-06	**-2.07**
acrB	APL_0587	acriflavine resistance protein	COG0841	V		CytoplasmicMembrane	667281..670400 [+]	6.80E-06	**-2.07**
ape2120	APL_1983	hypothetical protein				Cytoplasmic	2210703..2211335 [+]	1.04E-10	**-2.07**
tatB	APL_1986	sec-independent protein translocase-like protein TatB	COG1826	U			2213292..2213879 [+]	3.49E-06	**-2.06**
galT	APL_0994	galactose-1-phosphate uridylyltransferase	COG1085	C	2.7.7.12		1149428..1150477 [+]	7.61E-04	**-2.06**
guaB	APL_0593	inosine-5'-monophosphate dehydrogenase	COG0516	F	1.1.1.205	Cytoplasmic	677056..678519 [-]	2.12E-06	**-2.05**
tnaB	APL_1410	tTryptophan-specific transport protein	COG0814	E		CytoplasmicMembrane	1612209..1613411 [+]	1.69E-04	**-2.05**
ape1339	APL_1241	hypothetical protein	COG1966	T		CytoplasmicMembrane	1427864..1429450 [+]	4.66E-07	**-2.05**
menF	APL_1042	menaquinone-specific isochorismate synthase	COG1169	HQ	5.4.4.2		1209987..1211267 [-]	3.11E-06	**-2.04**
priA	APL_1032	primosomal protein N'	COG1198	L			1196311..1198527 [-]	8.55E-09	**-2.04**
perM	APL_0763	putative permease perM-like	COG0628	R		CytoplasmicMembrane	872142..873203 [+]	5.99E-05	**-2.04**
nusA	APL_0638	transcription elongation protein NusA	COG0195	K		Cytoplasmic	728746..730227 [+]	1.14E-03	**-2.04**
rpsM	APL_1781	30S ribosomal protein S13	COG0099	J		Cytoplasmic	2007635..2007991 [+]	4.50E-06	**-2.04**
rplW	APL_1762	50S ribosomal protein L23	COG0089	J			1998351..1998656 [+]	3.69E-04	**-2.03**
ape1101	APL_1016	putative nucleoside transporter	COG1972	F		CytoplasmicMembrane	1181936..1183204 [-]	4.18E-07	**-2.03**
rpsF	APL_1171	30S ribosomal protein S6	COG0360	J		Cytoplasmic	1346888..1347262 [-]	5.35E-08	**-2.03**
tyrP-B	APL_0597	tyrosine-specific transport protein 2	COG0814	E		CytoplasmicMembrane	682972..684189 [+]	3.87E-07	**-2.03**
cydC	APL_0826	transport ATP-binding protein CydC	COG4987	CO		CytoplasmicMembrane	954450..956117 [-]	6.31E-06	**-2.03**
glpA	APL_0379	anaerobic glycerol-3-phosphate dehydrogenase subunit A	COG0578	C	1.1.99.5	Cytoplasmic	431205..432890 [+]	4.85E-04	**-2.03**
ape0572	APL_0526	hypothetical protein	COG3683	R			600117..600758 [+]	1.31E-06	**-2.02**
mltA	APL_0816	membrane-bound lytic murein transglycosylase A precursor	COG2821	M	3.2.1.-		942188..943282 [-]	9.18E-06	**-2.02**
ape1646	APL_1534	putative transport system permease protein	COG0477	GEPR		CytoplasmicMembrane	1748274..1749338 [-]	3.24E-05	**-2.02**
tgt	APL_0723	queuine tRNA-ribosyltransferase	COG0343	J	2.4.2.29		831585..832733 [-]	1.32E-09	**-2.01**
ape2060	APL_1928	Hypothetical protein				CytoplasmicMembrane	2164766..2165350 [+]	8.49E-04	**-2.01**
ape1833	APL_1713	hypothetical protein				CytoplasmicMembrane	1944171..1946177 [-]	1.10E-06	**-2.01**
truD	APL_1926	tRNA pseudouridine synthase D				Cytoplasmic	2162989..2164002 [+]	4.94E-07	**-2.01**
glpQ	APL_0378	glycerophosphoryl diester phosphodiesterase	COG0584	C	3.1.4.46		429865..430953 [-]	6.58E-06	**-2.01**
rpsQ	APL_1769	30S ribosomal protein S17	COG0186	J		Cytoplasmic	2001485..2001739 [+]	1.36E-05	**-2.00**
ape1054	APL_0971	putative acyl CoA thioester hydrolase	COG1607	I	3.1.2.-		1125743..1126234 [-]	1.07E-05	**-2.00**

dnaQ	APL_1282	DNA polymerase III subunit epsilo	COG0847	L	2.7.7.7	Cytoplasmic	1473144..1473917 [-]	1.84E-07	**-2.00**
ape1091	APL_1006	hypothetical protein				CytoplasmicMembrane	1169734..1170603 [+]	4.41E-06	**-1.99**
ape1321	APL_1224	hypothetical protein	COG1553	P			1407172..1407522 [+]	2.18E-09	**-1.99**
secF	APL_1068	protein-export membrane protein SecF	COG0341	U		CytoplasmicMembrane	1236707..1237681 [+]	2.84E-09	**-1.98**
glgP	APL_0350	glycogen phosphorylase	COG0058	G	2.4.1.1	Cytoplasmic	390609..393113 [+]	1.27E-08	**-1.98**
rplC	APL_1760	50S ribosomal protein L3	COG0087	J			1997110..1997736 [+]	1.77E-06	**-1.98**
wecA	APL_1554	putative undecaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase	COG0472	M	2.7.8.-	CytoplasmicMembrane	1776632..1777705 [-]	6.49E-10	**-1.98**
cbiQ	APL_1621	putative ABC transport permease protein CbiQ	COG0619	P		CytoplasmicMembrane	1845340..1845957 [-]	1.34E-05	**-1.97**
ape0483	APL_0449	putative integral membrane protein	COG1757	C		CytoplasmicMembrane	516537..518054 [+]	5.85E-04	**-1.97**
ispZ	APL_0972	putative intracellular septation protein	COG2917	D		CytoplasmicMembrane	1126221..1126772 [-]	3.54E-07	**-1.97**
ape0637	APL_0586	putative RND efflux membrane fusion protein	COG0845	M			666063..667268 [+]	2.46E-05	**-1.97**
glgA	APL_0349	glycogen synthase	COG0297	G	2.4.1.21	Cytoplasmic	389143..390579 [+]	4.31E-10	**-1.97**
ubiD	APL_1439	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	COG0043	H	4.1.1.-		1645551..1647014 [-]	4.98E-07	**-1.97**
uraA	APL_0105	putative uracil permease	COG2233	F		CytoplasmicMembrane	120177..121406 [-]	4.17E-08	**-1.96**
asnS	APL_0675	asparaginyl-tRNA synthetase	COG0017	J	6.1.1.22	Cytoplasmic	770614..772017 [+]	3.57E-05	**-1.96**
cydD	APL_0827	transport ATP-binding protein CydD	COG4988	CO		CytoplasmicMembrane	956128..957870 [-]	4.78E-07	**-1.96**
wecB	APL_1552	UDP-N-acetylglucosamine 2-epimerase	COG0381	M	5.1.3.14	Cytoplasmic	1774599..1775678 [-]	4.27E-06	**-1.96**
rpsK	APL_1782	30S ribosomal protein S11	COG0100	J			2008008..2008397 [+]	1.65E-05	**-1.95**
pgsA	APL_0275	phosphatidylglycerophosphate synthase	COG0558	I	2.7.8.5	CytoplasmicMembrane	302222..302773 [+]	5.02E-07	**-1.95**
glpF	APL_0374	glycerol uptake facilitator protein	COG0580	G		CytoplasmicMembrane	423214..423996 [+]	1.26E-03	**-1.95**
ape1160	APL_1071	hypothetical protein	COG2252	R		CytoplasmicMembrane	1239071..1240372 [+]	2.58E-07	**-1.95**
potB	APL_0281	spermidine/putrescine transport system permease protein potB	COG1176	E		CytoplasmicMembrane	311260..312126 [+]	5.07E-09	**-1.94**
kpsF	APL_0387	arabinose-5-phosphate isomerase	COG0794	M		Cytoplasmic	440036..440971 [-]	5.22E-07	**-1.94**
rpoA	APL_1784	DNA-directed RNA polymerase alpha chain	COG0202	K	2.7.7.6	Cytoplasmic	2009118..2010104 [+]	1.19E-07	**-1.93**
rnfB	APL_0166	electron transport complex protein RnfB	COG2878	C		Cytoplasmic	184034..184624 [+]	2.84E-07	**-1.93**
aspS	APL_1149	aspartyl-tRNA synthetase	COG0173	J	6.1.1.12	Cytoplasmic	1324625..1326400 [+]	3.78E-07	**-1.92**
ape1377	APL_1277	hypothetical protein				Cytoplasmic	1469179..1469346 [-]	3.48E-02	**-1.92**
ubiB	APL_1984	Probable ubiquinone biosynthesis protein ubiB	COG0661	R			2211332..2212966 [+]	2.11E-05	**-1.92**
pulJ	APL_1887	hypothetical protein	COG4795	U		Cytoplasmic	2119962..2120495 [-]	4.90E-04	**-1.92**
dnaQ	APL_0182	DNA polymerase III, epsilon subunit and related 3'-5' exonucleases	COG0847	L	3.1.13.-		200701..201357 [+]	2.49E-05	**-1.92**
oxaA	APL_1424	inner membrane protein OxaA	COG0706	U		CytoplasmicMembrane	1629241..1630722 [+]	2.54E-07	**-1.92**
kefBC	APL_1053	glutathione-regulated potassium-efflux system protein	COG0475	P		CytoplasmicMembrane	1222558..1224423 [-]	2.58E-12	**-1.92**
bolA	APL_0235	stress-induced morphogen (activity unknown)	COG0271	T			253341..253652 [+]	4.12E-06	**-1.92**
ape1023	APL_0943	hypothetical adenine-specific methylase	COG2890	J	2.1.1.72	Cytoplasmic	1084230..1085174 [+]	2.12E-06	**-1.92**
ape1524	APL_1417	hypothetical protein				CytoplasmicMembrane	1619428..1619853 [+]	1.96E-06	**-1.92**
purE	APL_0659	phosphoribosylaminoimidazole carboxylase catalytic subunit	COG0041	F	4.1.1.21		752887..753381 [+]	4.05E-06	**-1.91**
ape0740	APL_0686	hypothetical protein				CytoplasmicMembrane	784609..785013 [-]	2.83E-02	**-1.91**
dnaB	APL_0713	replicative DNA helicase	COG0305	L	3.6.1.-		822616..824076 [-]	3.10E-06	**-1.91**
ape0188	APL_0179	hypothetical protein	COG1301	C			196507..199203 [-]	1.33E-09	**-1.91**
vacJ	APL_1918	lipoprotein VacJ-like precursor	COG2853	M			2151556..2152305 [+]	2.60E-05	**-1.91**
uppS	APL_0414	undecaprenyl pyrophosphate synthetase	COG0020	I	2.5.1.31		472284..472997 [-]	3.38E-05	**-1.91**
ape1043	APL_0961	putative hemolysin activation/secretion protein	COG2831	U		OuterMembrane	1112918..1113625 [-]	5.16E-05	**-1.91**
ape1012	APL_0932	putative HTH-type transcriptional regulator	COG1959	K		Cytoplasmic	1073111..1073563 [-]	7.58E-07	**-1.91**
rplQ	APL_1785	50S ribosomal protein L17	COG0203	J			2010213..2010527 [+]	2.33E-07	**-1.91**
glmU	APL_0588	bifunctional protein GlmU (Includes: UDP-N-acetylglucosamine pyrophosphorylase; Glucosamine-1-phosphate N-acetyltransferase)	COG1207	M	2.3.1.157 2.7.7.23	Cytoplasmic	670619..671992 [+]	4.51E-08	**-1.91**
nusB	APL_0201	transcription antitermination protein NusB	COG0781	K		Cytoplasmic	220866..221279 [+]	2.26E-06	**-1.91**
ape2011	APL_1882	hypothetical protein					2111784..2112536 [+]	2.02E-07	**-1.90**
cbiM	APL_1622	predicted ABC transport permease protein CbiM	COG0310	P		CytoplasmicMembrane	1845964..1846572 [-]	1.26E-06	**-1.90**

mepA	APL_0747	penicillin-insensitive murein endopeptidase precursor	COG3770	M	3.4.24.-		852612..853442 [-]	2.33E-05	**-1.90**
ape1920	APL_1792	Fe(III) dicitrate ABC transporter, permease	COG0609	P		CytoplasmicMembrane	2015459..2016340 [+]	1.90E-06	**-1.90**
kdtA	APL_1132	3-deoxy-D-manno-octulosonic-acid transferase	COG1519	M	2.-.-.-		1309230..1310510 [+]	2.45E-06	**-1.89**
prsA	APL_0775	ribose-phosphate pyrophosphokinase	COG0462	FE	2.7.6.1		891522..892472 [-]	2.17E-04	**-1.89**
ubiA	APL_0822	4-hydroxybenzoate octaprenyltransferase	COG0382	H	2.5.1.-	CytoplasmicMembrane	950519..951400 [-]	3.40E-06	**-1.89**
cyaA	APL_1054	adenylate cyclase	COG3072	F	4.6.1.1	Cytoplasmic	1224512..1227040 [-]	7.11E-10	**-1.89**
hisC	APL_0700	histidinol-phosphate aminotransferase 2	COG0079	E	2.6.1.9	Cytoplasmic	802505..803599 [-]	1.89E-06	**-1.88**
lgt	APL_1895	Prolipoprotein diacylglyceryl transferase	COG0682	M	2.4.99.-	CytoplasmicMembrane	2126510..2127304 [-]	5.82E-07	**-1.88**
ape1056	APL_0973	hypothetical protein				CytoplasmicMembrane	1126774..1127511 [-]	6.39E-08	**-1.88**
ape2069	APL_1936	hypothetical protein					2170294..2170437 [-]	3.25E-03	**-1.88**
cysQ	APL_1312	CysQ-like protein	COG1218	P			1503711..1504526 [-]	4.17E-08	**-1.88**
ape0790	APL_0730	hypothetical protein				Cytoplasmic	839028..839558 [+]	3.84E-04	**-1.87**
lysR	APL_1860	LysR protein	COG0583	K		Cytoplasmic	2091060..2091962 [+]	3.96E-04	**-1.87**
glpK	APL_0375	glycerol kinase	COG0554	C	2.7.1.30	Cytoplasmic	424031..425542 [+]	3.47E-07	**-1.87**
rpsN	APL_1772	30S ribosomal protein S14	COG0199	J			2003241..2003546 [+]	1.00E-06	**-1.87**
ykgE	APL_0446	putative dehydrogenase subunit	COG0247	C		Cytoplasmic	512616..513341 [-]	1.23E-05	**-1.87**
pyrH	APL_0569	uridylate kinase	COG0528	F	2.7.4.-	Cytoplasmic	642261..642974 [+]	3.70E-10	**-1.87**
ispB	APL_1995	octaprenyl-diphosphate synthase	COG0142	H	2.5.1.-	Cytoplasmic	2221125..2222120 [-]	3.83E-05	**-1.86**
ape2026	APL_1896	hypothetical protein	COG0730	R		CytoplasmicMembrane	2127357..2128148 [-]	6.23E-06	**-1.86**
rplV	APL_1765	50S ribosomal protein L22	COG0091	J		Cytoplasmic	1999812..2000144 [+]	2.13E-07	**-1.86**
ubiE	APL_1982	Ubiquinone/menaquinone biosynthesis methyltransferase ubiE	COG2226	H	2.1.1.-		2209901..2210677 [+]	2.58E-06	**-1.86**
aroA	APL_0699	3-phosphoshikimate 1-carboxyvinyltransferase	COG0128	E			801193..802491 [-]	3.94E-05	**-1.86**
ape1444	APL_1341	hypothetical protein				CytoplasmicMembrane	1541745..1542335 [-]	4.55E-04	**-1.85**
wecC	APL_1551	UDP-N-acetyl-D-mannosamine dehydrogenase	COG0677	M	1.1.1.-		1773249..1774520 [-]	1.02E-06	**-1.85**
secD	APL_1067	protein-export membrane protein SecD	COG0342	U		CytoplasmicMembrane	1234882..1236696 [+]	1.45E-06	**-1.85**
folE	APL_0903	GTP cyclohydrolase I	COG0302	H	3.5.4.16	Cytoplasmic	1045805..1046461 [-]	1.17E-04	**-1.85**
ushA	APL_0769	UshA precursor (Includes: UDP-sugar hydrolase; 5'-nucleotidase)	COG0737	F	3.1.3.5 3.6.1.45	Periplasmic	879630..881273 [+]	7.65E-06	**-1.84**
gyrB	APL_0821	DNA gyrase subunit B	COG0187	L	5.99.1.3		947874..950306 [-]	5.01E-07	**-1.84**
gpmB	APL_0230	phosphoglycerate mutase/fructose-2, 6-bisphosphatase	COG0406	G	5.4.2.1		249991..250623 [+]	5.99E-07	**-1.84**
ape1117	APL_1030	hypothetical protein	COG5039	GM		Cytoplasmic	1194351..1195316 [-]	6.54E-05	**-1.84**
gloA	APL_0181	lactoylglutathione lyase	COG0346	E	4.4.1.5		200154..200561 [+]	6.00E-08	**-1.83**
rpsD	APL_1783	30S ribosomal protein S4	COG0522	J			2008426..2009052 [+]	6.58E-07	**-1.83**
ape1667	APL_1553	hypothetical protein	COG3765	M			1775719..1776597 [-]	3.15E-05	**-1.83**
ape1299	APL_1203	hypothetical protein				Cytoplasmic	1386211..1386774 [+]	7.97E-08	**-1.83**
fabD	APL_1993	Malonyl CoA-acyl carrier protein transacylase (MCT)	COG0331	I	2.3.1.39		2219340..2220275 [-]	5.40E-07	**-1.83**
frdB	APL_1528	fumarate reductase iron-sulfur protein	COG0479	C	1.3.99.1		1741547..1742281 [-]	5.68E-07	**-1.83**
ape1302	APL_1205	putative glutathione S-transferase	COG0625	O			1387841..1388524 [-]	6.82E-09	**-1.83**
ape0716	APL_0664	hypothetical protein	COG1373	R			758756..759979 [-]	3.14E-06	**-1.83**
glpR	APL_0823	glycerol-3-phosphate regulon repressor	COG1349	KG			951400..952161 [-]	8.67E-05	**-1.83**
ubiX	APL_0732	3-octaprenyl-4-hydroxybenzoate carboxy-lyase	COG0163	H	4.1.1.-	Cytoplasmic	840034..840606 [+]	1.79E-04	**-1.83**
ape0879	APL_0814	hypothetical protein	COG0500	QR		Cytoplasmic	940675..941637 [+]	7.20E-04	**-1.83**
potA	APL_0280	spermidine/putrescine import ATP-binding protein PotA	COG3842	E	3.6.3.31	Cytoplasmic	310158..311273 [+]	9.42E-10	**-1.82**
yhiR	APL_0317	hypothetical protein	COG2961	R			353973..354815 [+]	2.10E-07	**-1.82**
ape1084	APL_0999	hypothetical protein					1161732..1162208 [-]	8.61E-07	**-1.82**
ape0468	APL_0438	hypothetical protein	COG2256	L			494439..495779 [+]	1.94E-06	**-1.82**
truA	APL_0902	tRNA pseudouridine synthase A	COG0101	J	5.4.99.12		1044941..1045726 [-]	1.22E-03	**-1.82**
ape1650	APL_1538	predicted acetyltransferase	COG0454	KR			1752542..1753009 [-]	8.56E-08	**-1.82**
glpB	APL_0380	anaerobic glycerol-3-phosphate dehydrogenase subunit B	COG3075	E	1.1.99.5		432880..434166 [+]	6.29E-05	**-1.81**
pulG	APL_1888	hypothetical protein	COG2165	NU			2120620..2121150 [-]	1.73E-04	**-1.81**

ape1020	APL_0940	hypothetical protein					1080982..1081641 [-]	3.57E-07	**-1.81**
cpdB	APL_0646	2',3'-cyclic-nucleotide 2'-phosphodiesterase precursor	COG0737	F	3.1.4.16	Periplasmic	739513..741489 [+]	8.99E-04	**-1.80**
rseC	APL_0396	putative sigma-E factor regulatory protein	COG3086	T			450532..450960 [+]	6.86E-07	**-1.80**
ape1053	APL_0970	hypothetical protein				Cytoplasmic	1125450..1125716 [-]	1.09E-07	**-1.80**
ape1459	APL_1356	putative sodium/calcium exchange protein	COG0530	P		CytoplasmicMembrane	1553553..1554512 [+]	1.29E-08	**-1.80**
rplR	APL_1775	50S ribosomal protein L18	COG0256	J		Cytoplasmic	2004540..2004893 [+]	2.33E-06	**-1.79**
ape0892	APL_0825	hypothetical protein	COG2915	R			953653..954285 [-]	1.65E-05	**-1.79**
mukF	APL_0579	chromosome partition protein MukF	COG3006	D		Cytoplasmic	655340..656671 [+]	3.61E-06	**-1.79**
yajC	APL_1066	preprotein translocase subunit YajC	COG1862	U			1234526..1234822 [+]	4.08E-07	**-1.79**
rpsL	APL_1401	30S ribosomal protein S12	COG0048	J			1602612..1602986 [-]	5.86E-07	**-1.79**
rplD	APL_1761	50S ribosomal protein L4	COG0088	J			1997752..1998354 [+]	8.28E-08	**-1.78**
rpoC	APL_1728	DNA-directed RNA polymerase beta' chain	COG0086	K	2.7.7.6	Cytoplasmic	1961382..1965620 [+]	8.48E-06	**-1.78**
lysS	APL_0809	lysyl-tRNA synthetase	COG1190	J	6.1.1.6	Cytoplasmic	930728..932230 [-]	2.19E-05	**-1.78**
wecD	APL_1550	putative TDP-D-fucosamine acetyltransferase	COG0454	KR			1772626..1773249 [-]	1.09E-05	**-1.78**
ape0377	APL_0356	hypothetical protein					399116..399502 [-]	1.42E-04	**-1.78**
mtlA	APL_1630	PTS system mannitol-specific EIICBA component [Includes: Mannitol permease IIC component; Mannitol-specific phosphotransferase enzyme IIB component; Mannitol-specific phosphotransferase enzyme IIA component]	COG2213	G	2.7.1.69	CytoplasmicMembrane	1853283..1855172 [-]	2.43E-04	**-1.78**
ape0822	APL_0762	hypothetical protein	COG0500	QR		Cytoplasmic	871328..872083 [+]	1.79E-05	**-1.77**
tyrR	APL_0797	transcriptional regulatory protein TyrR	COG3283	KE		Cytoplasmic	918871..920019 [-]	4.63E-07	**-1.77**
ulaA	APL_1714	ascorbate-specific permease IIC component UlaA				CytoplasmicMembrane	1946399..1947754 [-]	5.63E-04	**-1.77**
ape0239	APL_0228	hypothetical protein	COG1253	R		CytoplasmicMembrane	247952..249247 [-]	2.15E-08	**-1.77**
glpT	APL_1835	Glycerol-3-phosphate transporter	COG2271	G		CytoplasmicMembrane	2058079..2059236 [-]	6.65E-06	**-1.77**
sapC	APL_0794	peptide transport system permease protein SapC	COG4171	V		CytoplasmicMembrane	915325..916212 [-]	1.08E-08	**-1.77**
purB	APL_0824	adenylosuccinate lyase	COG0015	F	4.3.2.2		952229..953596 [-]	4.55E-07	**-1.77**
ape2077	APL_1943	ADP-ribose pyrophosphatase	COG0494	LR	3.6.1.13	Cytoplasmic	2175500..2176123 [-]	3.36E-09	**-1.77**
selD	APL_0327	selenide, water dikinase	COG0709	E	2.7.9.3	Cytoplasmic	364716..365633 [+]	3.84E-03	**-1.76**
ape1216	APL_1126	putative micrococcal nuclease (thermonuclease)-like	COG1525	L			1302072..1302770 [-]	4.39E-07	**-1.76**
ape0288	APL_0274	hypothetical protein	COG0670	R		CytoplasmicMembrane	301375..302040 [-]	9.71E-07	**-1.76**
coaE	APL_0876	dephospho-CoA kinase	COG0237	H	2.7.1.24	Cytoplasmic	1016712..1017350 [-]	4.31E-04	**-1.76**
ape1128	APL_1041	hypothetical protein				CytoplasmicMembrane	1209040..1209948 [+]	5.14E-03	**-1.76**
argC	APL_0242	N-acetyl-gamma-glutamyl-phosphate reductase (AGPR)	COG0002	E	1.2.1.38		259949..260893 [+]	2.52E-06	**-1.76**
mutT	APL_0328	putative NTP pyrophosphohydrolase (MutT/Nudix family)	COG0494	LR	3.6.-.-	Cytoplasmic	365638..366093 [+]	1.68E-05	**-1.75**
rpsH	APL_1773	30S ribosomal protein S8	COG0096	J			2003583..2003975 [+]	3.77E-07	**-1.75**
zwf	APL_1311	glucose-6-phosphate 1-dehydrogenase	COG0364	G	1.1.1.49		1502152..1503639 [-]	1.80E-07	**-1.75**
rplT	APL_0225	50S ribosomal protein L20	COG0292	J			246631..246984 [+]	2.13E-04	**-1.75**
sapF	APL_1249	peptide transport system ATP-binding protein SapF	COG4167	V			1435370..1436170 [-]	4.83E-07	**-1.75**
ape1106	APL_1021	alpha-glucosidase 2	COG1501	G	3.2.1.20		1186618..1188096 [+]	3.54E-08	**-1.74**
leuD	APL_0138	3-isopropylmalate dehydratase small subunit	COG0066	E	4.2.1.33	Cytoplasmic	153561..154163 [-]	1.34E-10	**-1.74**
trmE	APL_1490	tRNA modification GTPase TrmE	COG0486	R		Cytoplasmic	1705081..1706439 [+]	6.48E-05	**-1.74**
hflK	APL_1077	protein HflK	COG0330	O	3.4.-.-		1245823..1247013 [-]	1.73E-05	**-1.74**
nudH	APL_1897	Probable (di)nucleoside polyphosphate hydrolase	COG0494	LR	3.6.1.-		2128145..2128765 [-]	4.10E-07	**-1.74**
malT	APL_1233	HTH-type transcriptional regulator MalT	COG2909	K			1415184..1417901 [-]	1.37E-05	**-1.74**
aroG	APL_0620	phospho-2-dehydro-3-deoxyheptonate aldolase	COG0722	E	2.5.1.54	Cytoplasmic	707965..709038 [+]	7.95E-07	**-1.73**
ape1716	APL_1601	hypothetical protein				Cytoplasmic	1828414..1828728 [-]	5.32E-03	**-1.73**
glnE	APL_0969	glutamate-ammonia-ligase adenylyltransferase	COG1391	OT	2.7.7.42	Cytoplasmic	1122496..1125375 [-]	2.26E-04	**-1.73**
suhB	APL_0206	Inositol-1-monophosphatase (IMPase)	COG0483	G	3.1.3.25	Cytoplasmic	224692..225504 [+]	1.27E-07	**-1.73**
purM	APL_1095	phosphoribosylformylglycinamidine cyclo-ligase	COG0150	F	6.3.3.1		1266330..1267367 [-]	3.24E-05	**-1.73**
ape1487	APL_1382	hypothetical protein				Cytoplasmic	1583783..1584124 [-]	2.24E-05	**-1.72**

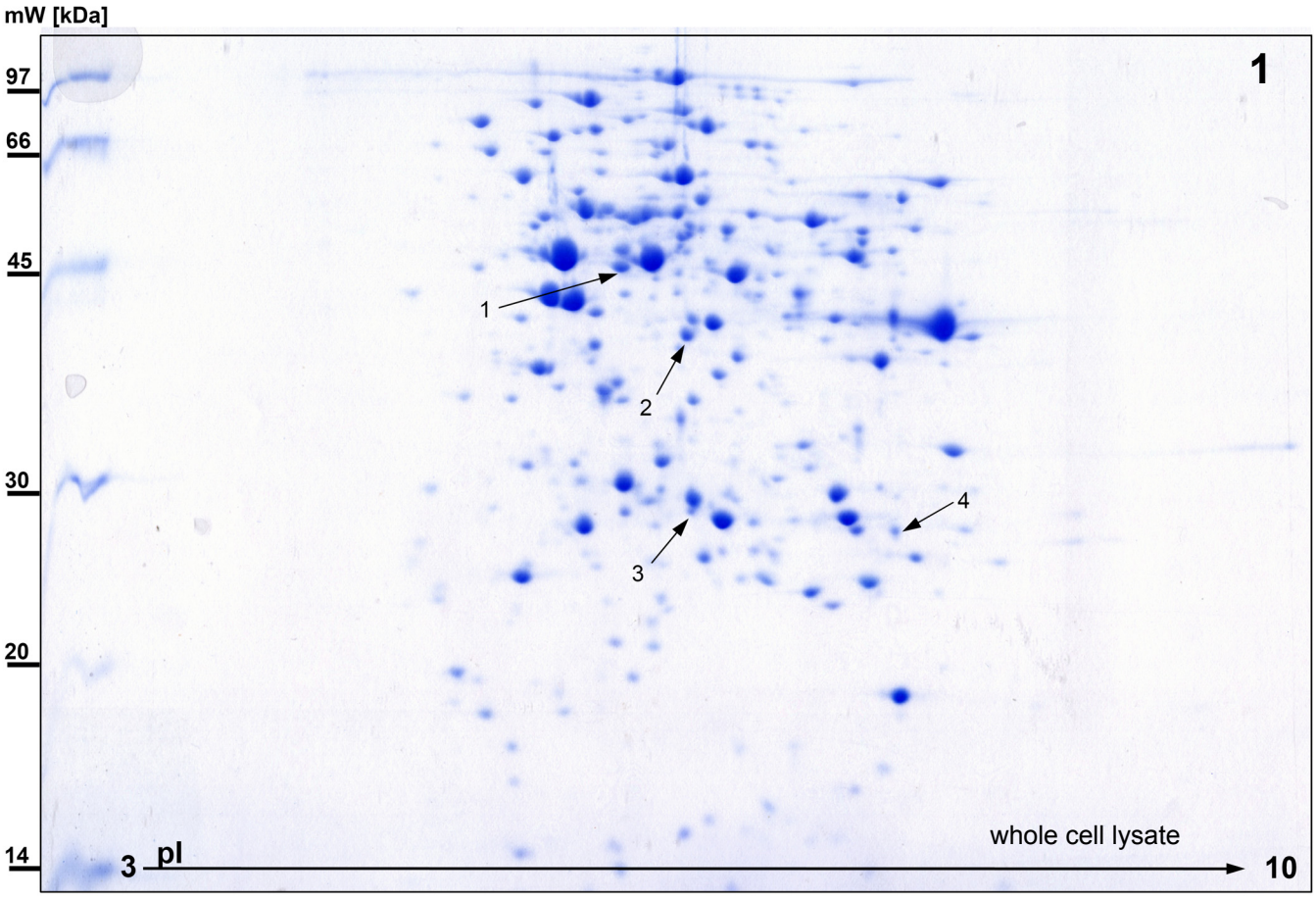
rpsG	APL_1400	30S ribosomal protein S7	COG0049	J			1602005..1602475 [-]	1.64E-06	**-1.72**
arcB	APL_1317	ornithine carbamoyltransferase, catabolic	COG0078	E	2.1.3.3	Cytoplasmic	1508919..1509923 [+]	5.56E-06	**-1.72**
rbsK	APL_1673	ribokinase	COG0524	G	2.7.1.15	Cytoplasmic	1898947..1899858 [+]	1.00E-06	**-1.72**
ape1918	APL_1790	Protein HI0396				Cytoplasmic	2012932..2014125 [-]	1.86E-05	**-1.72**
hflX	APL_1962	GTP-binding protein hflX	COG2262	R		Cytoplasmic	2192241..2193464 [+]	3.36E-03	**-1.72**
ape0053	APL_0051	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase	COG1778	R	3.1.3.45	Cytoplasmic	57311..57847 [-]	4.04E-08	**-1.71**
hflC	APL_1076	protein HflC	COG0330	O	3.4.-.-	Cytoplasmic	1244933..1245820 [-]	1.12E-09	**-1.71**
pflA	APL_1035	pyruvate formate-lyase 1-activating enzyme	COG1180	O	1.97.1.4	Cytoplasmic	1202068..1202805 [-]	2.34E-06	**-1.71**
mutS	APL_1602	DNA mismatch repair protein MutS	COG0249	L			1828954..1831548 [-]	1.37E-03	**-1.71**
msrB	APL_0433	methionine sulfoxide reductase B	COG0229	O	1.8.4.12	Cytoplasmic	489902..490297 [-]	1.55E-04	**-1.71**
mipB	APL_0062	transaldolase	COG0176	G	2.2.1.2		67907..68854 [-]	2.61E-06	**-1.71**
ape0824	APL_0764	hypothetical protein					873233..874162 [+]	6.33E-06	**-1.70**
nqrB	APL_0151	Na(+)-translocating NADH-quinone reductase subunit B	COG1805	C	1.6.5.-	CytoplasmicMembrane	169440..170672 [+]	2.35E-05	**-1.70**
cbiO	APL_1620	predicted ABC transport ATP-binding protein CbiO	COG1122	P			1844688..1845317 [-]	9.08E-06	**-1.70**
ansB	APL_0660	probable L-asparaginase	COG0252	EJ	3.5.1.1	Cytoplasmic	753391..754356 [+]	1.27E-05	**-1.70**
fabG	APL_1992	3-oxoacyl-[acyl-carrier-protein] reductase (3-ketoacyl-acyl carrier protein reductase)	COG1028	IQR		Cytoplasmic	2218496..2219221 [-]	6.20E-07	**-1.70**
ape1285	APL_1190	putative transcriptional regulator LysR-like	COG0583	K		Cytoplasmic	1368533..1369432 [-]	2.74E-07	**-1.70**
murB	APL_1851	UDP-N-acetylenolpyruvoylglucosamine reductase	COG0812	M	1.1.1.158	Cytoplasmic	2081993..2083027 [-]	3.74E-06	**-1.70**
rnpA	APL_1939	ribonuclease P	COG0594	J	3.1.26.5		2172077..2172379 [+]	3.96E-04	**-1.70**
hcaT	APL_0159	putative 3-phenylpropionic acid transporter	COG0477	GEPR		CytoplasmicMembrane	176876..178030 [-]	9.91E-07	**-1.70**
greB	APL_0386	transcription elongation factor GreB	COG0782	K		Cytoplasmic	439538..440026 [-]	6.61E-09	**-1.70**
dgkA	APL_0768	diacylglycerol kinase	COG0818	M	2.7.1.107	CytoplasmicMembrane	879187..879549 [+]	3.67E-05	**-1.70**
nhaA	APL_1947	Na(+)/H(+) antiporter 1 (Sodium/proton antiporter 1)	COG3004	P		CytoplasmicMembrane	2177937..2179121 [+]	3.28E-06	**-1.69**
tyrS	APL_1476	tyrosyl-tRNA synthetase	COG0162	J	6.1.1.1	Cytoplasmic	1690213..1691403 [-]	7.39E-05	**-1.69**
ape0724	APL_0672	hypothetical protein	COG3663	L			766607..767197 [-]	2.57E-04	**-1.69**
rpoB	APL_1727	DNA-directed RNA polymerase beta chain	COG0085	K	2.7.7.6	Cytoplasmic	1957228..1961256 [+]	4.08E-04	**-1.69**
hemL	APL_1555	glutamate-1-semialdehyde 2,1-aminomutase	COG0001	H	5.4.3.8	CytoplasmicMembrane	1778004..1779284 [+]	6.29E-04	**-1.69**
ccmA	APL_1372	cytochrome c biogenesis ATP-binding export protein CcmA	COG4133	O	3.6.3.41	CytoplasmicMembrane	1568850..1569485 [-]	2.48E-06	**-1.69**
ape1300	APL_1204	esterase	COG0627	R	3.1.1.-		1386848..1387705 [-]	2.87E-06	**-1.69**
rplB	APL_1763	50S ribosomal protein L2	COG0090	J			1998677..1999498 [+]	6.95E-06	**-1.69**
namA	APL_1191	NADPH dehydrogenase	COG1902	C		Cytoplasmic	1369523..1370584 [+]	1.37E-03	**-1.68**
cbiL	APL_1623	putative cytoplasmic membrane protein CbiL					1846573..1847055 [-]	1.32E-05	**-1.68**
ape0349	APL_0329	2-octaprenyl-6-methoxyphenol hydroxylase	COG0654	HC	1.14.13.-		366103..367302 [+]	1.33E-08	**-1.68**
ape0901	APL_0833	hypothetical protein	COG1279	R		CytoplasmicMembrane	966885..967520 [-]	6.25E-04	**-1.68**
ape0171	APL_0162	putative phosphatase	COG0637	R		Cytoplasmic	179938..180543 [+]	1.36E-10	**-1.68**
ape1322	APL_1225	hypothetical protein	COG2081	R		CytoplasmicMembrane	1407522..1408241 [+]	2.23E-06	**-1.68**
rdgC	APL_0161	recombination-associated protein RdgC	COG2974	L		Cytoplasmic	179020..179928 [+]	2.63E-06	**-1.68**
ape1345	APL_1247	hypothetical protein				CytoplasmicMembrane	1434344..1434637 [-]	2.41E-05	**-1.68**
ape0876	APL_0811	hypothetical protein				Cytoplasmic	934070..935833 [+]	3.40E-12	**-1.68**
rraA	APL_1901	Regulator of ribonuclease activity A	COG0684	H		Cytoplasmic	2131532..2132029 [-]	1.05E-03	**-1.68**
eda	APL_1018	putative KHG/KDPG aldolase [Includes: 4-hydroxy-2-oxoglutarate aldolase; 2-dehydro-3-deoxy-phosphogluconate aldolase]	COG0800	G	4.1.2.14	Cytoplasmic	1184113..1184754 [-]	7.96E-07	**-1.68**
parC	APL_0578	DNA topoisomerase 4 subunit A	COG0188	L	5.99.1.-	Cytoplasmic	652954..655200 [+]	1.35E-06	**-1.67**
ape0927	APL_0858	hypothetical protein	COG0665	E			997904..999919 [-]	2.98E-04	**-1.67**
lpxL	APL_0900	lipid A biosynthesis lauroyl acyltransferase	COG1560	M	2.3.1.-	CytoplasmicMembrane	1043459..1044400 [-]	4.35E-05	**-1.67**
ompP2A	APL_0006	outer membrane protein P2	COG3203	M		OuterMembrane	6134..7264 [-]	4.01E-04	**-1.67**
vacB	APL_1478	ribonuclease R	COG0557	K	3.1.-.-		1692326..1694683 [-]	2.04E-07	**-1.67**
ape0920	APL_0851	ABC transporter, ATP-binding subunit	COG4608	E			988016..988822 [+]	7.66E-06	**-1.67**
hemD	APL_1009	putative uroporphyrinogen-III synthase	COG1587	H	4.2.1.75		1173273..1174037 [-]	3.89E-07	**-1.67**

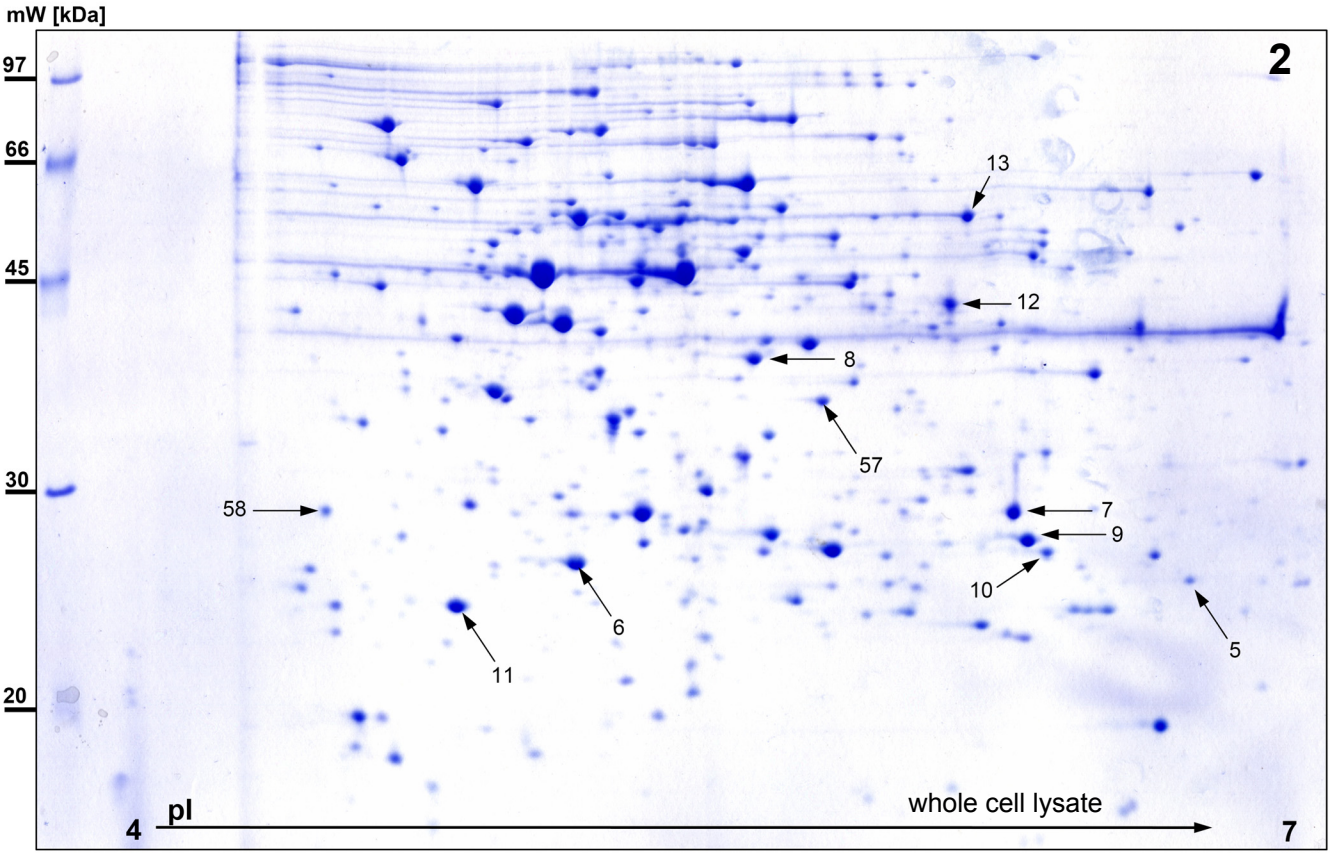
mutL	APL_1958	DNA mismatch repair protein mutL	COG0323	L			2187521..2189389 [+]	4.27E-06	**-1.66**
ape1591	APL_1482	hybrid peroxiredoxin HyPrx5	COG0678	O			1696909..1697640 [+]	9.22E-07	**-1.66**
pepP	APL_0663	Xaa-Pro aminopeptidase	COG0006	E	3.4.11.9	Cytoplasmic	757061..758347 [-]	3.22E-07	**-1.66**
ape1488	APL_1383	tRNA (guanine-N(7)-)-methyltransferase	COG0220	R	2.1.1.33	Cytoplasmic	1584180..1584932 [-]	1.06E-07	**-1.66**
dusA	APL_0146	tRNA-dihydrouridine synthase A	COG0042	J	1.-.-.-	Cytoplasmic	162938..163915 [-]	1.29E-04	**-1.66**
ackA	APL_0645	acetate kinase	COG0282	C	2.7.2.1	Cytoplasmic	738008..739216 [-]	9.77E-06	**-1.66**
malK	APL_1236	maltose/maltodextrin import ATP-binding protein MalK	COG3839	G	3.6.3.19	Cytoplasmic	1420312..1421451 [-]	3.19E-03	**-1.66**
sapA	APL_0796	peptide transport periplasmic protein SapA precursor	COG4166	E			917164..918861 [-]	8.86E-06	**-1.65**
ape1522	APL_1415	hypothetical protein				CytoplasmicMembrane	1618412..1618825 [-]	7.14E-05	**-1.65**
rne	APL_1436	ribonuclease E	COG1530	J	3.1.4.-	Cytoplasmic	1640346..1643333 [-]	4.62E-10	**-1.65**
mtlD	APL_1629	mannitol-1-phosphate 5-dehydrogenase	COG0246	G	1.1.1.17	Cytoplasmic	1852057..1853196 [-]	1.15E-04	**-1.65**
putP	APL_0107	sodium/proline symporter	COG0591	ER		CytoplasmicMembrane	125274..126788 [-]	3.24E-04	**-1.65**
ape1662	APL_1548	hypothetical protein	COG2244	R		CytoplasmicMembrane	1770236..1771486 [-]	4.18E-06	**-1.65**
ape0635	APL_0584	hypothetical protein					665021..665242 [-]	8.93E-04	**-1.65**
cdsA	APL_0413	phosphatidate cytidylyltransferase	COG0575	I	2.7.7.41	CytoplasmicMembrane	471390..472259 [-]	4.30E-06	**-1.65**
bioA	APL_0942	adenosylmethionine-8-amino-7-oxononanoate aminotransferase	COG0161	H	2.6.1.62		1082841..1084139 [-]	1.99E-06	**-1.64**
rplE	APL_1771	50S ribosomal protein L5	COG0094	J		Cytoplasmic	2002690..2003229 [+]	1.99E-06	**-1.64**
recB	APL_0370	exodeoxyribonuclease V beta chain	COG1074	L	3.1.11.5		416034..419642 [+	6.35E-08	**-1.64**
ape1130	APL_1043	probable aminotransferase	COG0436	E	2.6.1.1	Cytoplasmic	1211431..1212648 [+]	1.17E-05	**-1.64**
nqrF	APL_0155	Na(+)-translocating NADH-quinone reductase subunit F	COG2871	C	1.6.5.-		172680..173909 [+]	2.46E-08	**-1.63**
ape0619	APL_0568	hypothetical protein	COG2194	R		CytoplasmicMembrane	640596..642149 [-]	5.25E-05	**-1.63**
ape1588	APL_1479	thioredoxin-like protein	COG0526	OC			1694751..1695266 [-]	1.90E-06	**-1.63**
ape1168	APL_1079	putative purine permease	COG2233	F		CytoplasmicMembrane	1247641..1248933 [-]	1.25E-04	**-1.62**
ntpA	APL_1150	dATP pyrophosphohydrolase	COG0494	LR	3.6.1.-		1326434..1326904 [+]	2.72E-07	**-1.62**
rplL	APL_1721	50S ribosomal protein L7/L12	COG0222	J			1951568..1951936 [+]	1.58E-06	**-1.62**
holC	APL_1505	DNA polymerase III subunit chi	COG2927	L	2.7.7.7		1721997..1722434 [-]	3.22E-05	**-1.62**
rbsC	APL_1671	ribose transport system permease protein RbsC	COG1172	G			1897019..1897978 [+]	6.70E-07	**-1.62**
bioC	APL_0939	biotin synthesis protein	COG0500	QR		Cytoplasmic	1080224..1080994 [-]	6.10E-08	**-1.62**
hemB	APL_1988	delta-aminolevulinic acid dehydratase	COG0113	H	4.2.1.24	Cytoplasmic	2214675..2215694 [+]	4.72E-05	**-1.62**
ape1383	APL_1283	ABC transporter ATP-binding protein	COG0488	R		CytoplasmicMembrane	1474004..1475674 [-]	2.80E-03	**-1.62**
ape2078	APL_1944	Predicted membrane protein	COG3223			CytoplasmicMembrane	2176133..2176552 [-]	6.31E-03	**-1.62**
kdgK	APL_1019	2-dehydro-3-deoxygluconokinase	COG0524	G	2.7.1.45		1184769..1185713 [-]	9.01E-09	**-1.62**
ape0396	APL_0373	hypothetical protein					422376..422978 [+]	1.95E-05	**-1.61**
ape0156	APL_0147	ribonucleoside-diphosphate reductase beta chain	COG0208	F	1.17.4.1	Cytoplasmic	164268..165245 [+]	6.30E-05	**-1.61**
utp	APL_1619	urea transport protein ApUT	COG4413	E		CytoplasmicMembrane	1843761..1844576 [-]	8.97E-06	**-1.61**
plsC	APL_1488	1-acyl-sn-glycerol-3-phosphate acyltransferase	COG0204	I	2.3.1.51	CytoplasmicMembrane	1703581..1704315 [-]	1.30E-05	**-1.61**
apbE	APL_0156	thiamine biosynthesis lipoprotein ApbE precursor	COG1477	H			174116..175147 [+]	3.92E-05	**-1.61**
ape0797	APL_0737	hypothetical protein	COG2956	G		Cytoplasmic	842999..844195 [-]	1.67E-06	**-1.61**
atpA	APL_1648	ATP synthase subunit alpha	COG0056	C	3.6.3.14		1876081..1877622 [-]	1.06E-02	**-1.60**
mglA	APL_1419	galactoside transport ATP-binding protein MglA	COG1129	G	3.6.3.17	CytoplasmicMembrane	1620951..1622450 [-]	2.05E-06	**-1.60**
artQ	APL_1352	arginine transport system permease protein ArtQ	COG4215	E		CytoplasmicMembrane	1550608..1551279 [	5.29E-05	**-1.60**
ape0856	APL_0792	hypothetical protein					913424..914191 [-]	3.69E-09	**-1.60**
pckA	APL_0800	phosphoenolpyruvate carboxykinase [ATP]	COG1866	C	4.1.1.49	Cytoplasmic	922121..923731 [-]	1.86E-03	**-1.60**
fabH	APL_1384	3-oxoacyl-[acyl-carrier-protein] synthase 3	COG0332	I	2.3.1.41		1584980..1585930 [-]	1.08E-04	**-1.60**
ape2135	APL_1998	Hypothetical protein				CytoplasmicMembrane	2224143..2224556 [+]	1.82E-06	**-1.60**
xthA	APL_0977	exodeoxyribonuclease III	COG0708	L	3.1.11.2	Cytoplasmic	1130902..1131705 [+]	3.61E-05	**-1.60**
ape0502	APL_0468	probable NADP-dependent dehydrogenase	COG4221	R	1.-.-.-	Cytoplasmic	539525..540274 [+]	2.30E-04	**-1.60**
ape1353	APL_1254	hypothetical protein	COG0471	P		CytoplasmicMembrane	1441994..1443385 [-]	5.00E-08	**-1.60**
glpX	APL_0624	fructose-1,6-bisphosphatase class II GlpX	COG1494	G	3.1.3.11		711318..712316 [+]	2.34E-04	**-1.59**

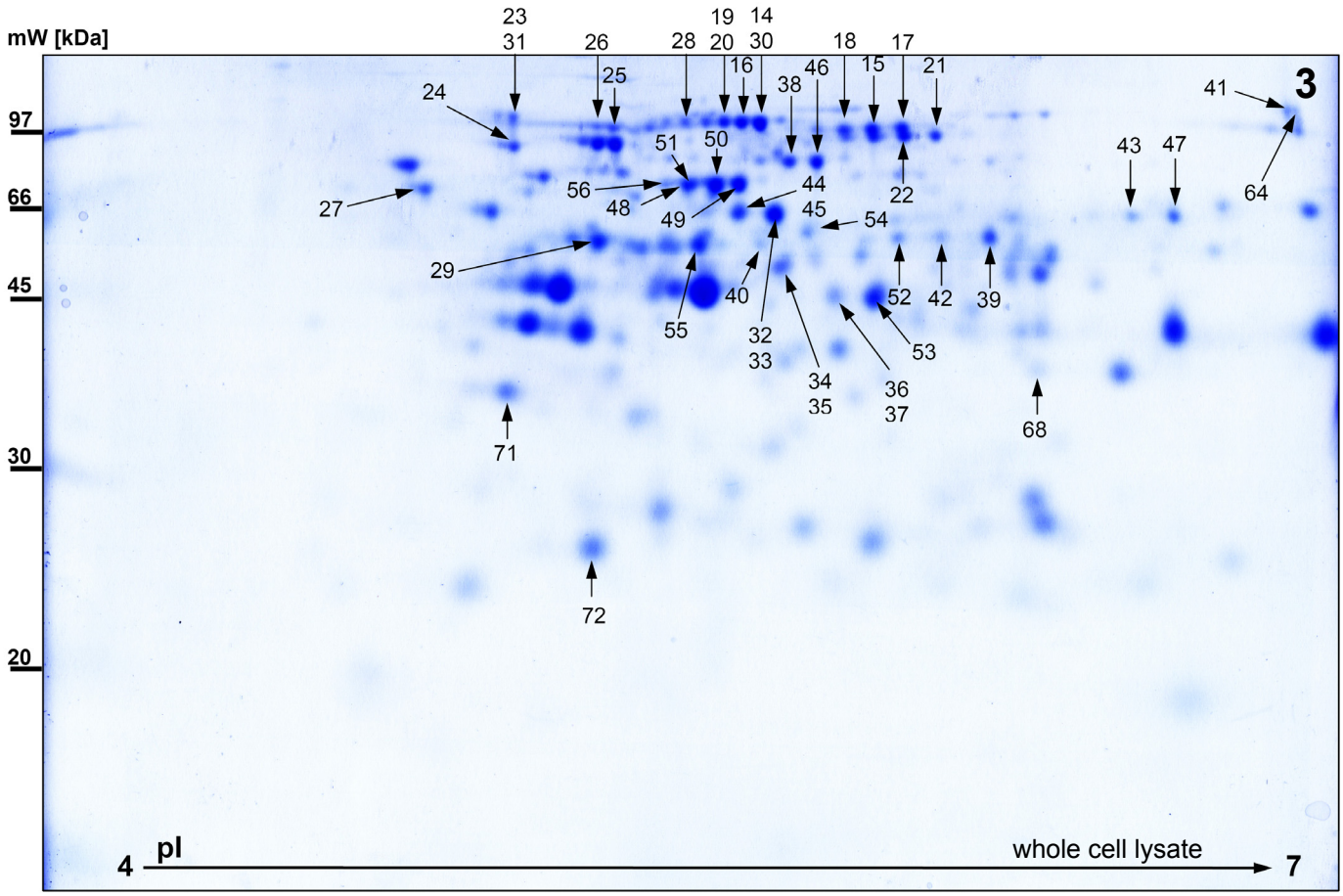
ape0556	APL_0512	hypothetical protein					581915..582217 [+]	7.64E-03	**-1.59**
ape0969	APL_0898	putative oxalate/formate antiporter	COG0477	GEPR		CytoplasmicMembrane	1040777..1042315 [+]	9.87E-08	**-1.59**
ape1323	APL_1226	hypothetical protein	COG2081	R		Cytoplasmic	1408272..1408709 [+]	2.37E-06	**-1.59**
fdxH	APL_0894	formate dehydrogenase, iron-sulfur subunit	COG0437	C		CytoplasmicMembrane	1036582..1037481 [+]	6.49E-06	**-1.59**
icc	APL_1942	protein Icc-like	COG1409	R		Cytoplasmic	2174466..2175293 [-]	1.60E-06	**-1.59**
ppa	APL_1899	Inorganic pyrophosphatase	COG0221	C	3.6.1.1	Cytoplasmic	2130174..2130701 [-]	7.59E-05	**-1.58**
ape1810	APL_1692	putative ABC transporter, permease protein, amino acid	COG0765	E		CytoplasmicMembrane	1922877..1923545 [+]	6.63E-06	**-1.58**
ape1220	APL_1130	putative protease	COG0826	O	3.4.-.-	Cytoplasmic	1306339..1307736 [+]	5.85E-05	**-1.58**
gor	APL_1243	glutathione reductase	COG1249	C	1.8.1.7	Cytoplasmic	1430369..1431739 [-]	2.50E-07	**-1.58**
rpsC	APL_1766	30S ribosomal protein S3	COG0092	J			2000163..2000870 [+]	4.08E-04	**-1.58**
rplP	APL_1767	50S ribosomal protein L16	COG0197	J			2000884..2001294 [+]	3.03E-05	**-1.58**
prmA	APL_1537	ribosomal protein L11 methyltransferase	COG2264	J	2.1.1.-	Cytoplasmic	1751577..1752458 [-]	6.72E-05	**-1.58**
glnD	APL_0598	uridylyltransferase	COG2844	O	2.7.7.59	Cytoplasmic	684296..686848 [+]	2.64E-06	**-1.58**
ribE	APL_0383	riboflavin synthase alpha chain	COG0307	H	2.5.1.9	Cytoplasmic	436964..437611 [+]	2.11E-06	**-1.57**
poxA	APL_1530	putative lysyl-tRNA synthetase	COG2269	J	6.1.1.6	Cytoplasmic	1744436..1745419 [+]	6.27E-06	**-1.57**
frdC	APL_1527	fumarate reductase subunit C	COG3029	C	1.3.99.1	CytoplasmicMembrane	1741146..1741538 [-]	1.07E-06	**-1.57**
uxaC	APL_1020	uronate isomerase	COG1904	G	5.3.1.12		1185726..1186316 [-]	5.52E-06	**-1.57**
cydA	APL_0297	cytochrome oxidase subunit 1	COG1271	C	1.10.3.-	CytoplasmicMembrane	329378..330925 [+]	3.91E-07	**-1.57**
glgB	APL_0346	1,4-alpha-glucan branching enzyme	COG0296	G	2.4.1.18	Cytoplasmic	383383..385716 [+]	5.49E-07	**-1.57**
cvpA	APL_0424	colicin V production protein	COG1286	R		CytoplasmicMembrane	481167..481670 [+]	1.85E-08	**-1.56**
ape1715	APL_1600	hypothetical protein					1827924..1828391 [-]	2.15E-02	**-1.56**
cydB	APL_0298	cytochrome oxidase subunit 2	COG1294	C	1.10.3.-	CytoplasmicMembrane	330942..332078 [+]	9.53E-05	**-1.56**
dsbD	APL_1359	thiol:disulfide interchange protein DsbD precursor	COG4232	OC	1.8.1.8	CytoplasmicMembrane	1555432..1557183 [+]	1.94E-03	**-1.56**
ape1751	APL_1636	hypothetical protein	COG0621	J		Cytoplasmic	1861550..1862881 [-]	1.78E-02	**-1.56**
ribH	APL_0385	6,7-dimethyl-8-ribityllumazine synthase	COG0054	H			438988..439449 [+]	2.89E-05	**-1.56**
ape1303	APL_1206	plasmid stability-like protein	COG1487	R			1388658..1389077 [-]	2.52E-06	**-1.56**
leuB	APL_0432	3-isopropylmalate dehydrogenase	COG0473	CE	1.1.1.85	Cytoplasmic	488696..489778 [+]	3.88E-08	**-1.55**
glgC	APL_0348	glucose-1-phosphate adenylyltransferase	COG0448	G	2.7.7.27		387724..389040 [+]	8.96E-05	**-1.55**
lnt	APL_0371	apolipoprotein N-acyltransferase	COG0815	M	2.3.1.-	CytoplasmicMembrane	419720..421231 [-]	1.81E-06	**-1.55**
ftnA	APL_1069	ferritin-like protein 1	COG1528	P		Cytoplasmic	1237828..1238334 [+]	9.06E-08	**-1.55**
secY	APL_1779	Preprotein translocase secY subunit	COG0201	U		CytoplasmicMembrane	2006037..2007359 [+]	1.72E-03	**-1.55**
ape0557	APL_0513	putative phage tail component					582220..582747 [+]	1.88E-02	**-1.55**
mlc	APL_0615	putative transcriptional repressor of carbohydrate metabolism	COG1940	KG			700861..702051 [-]	2.56E-03	**-1.55**
ape1116	APL_1029	hypothetical protein	COG2244	R		CytoplasmicMembrane	1193165..1194310 [-]	7.31E-07	**-1.55**
engA	APL_0403	GTP-binding protein EngA	COG1160	R		Cytoplasmic	457528..459048 [-]	6.07E-03	**-1.55**
gnd	APL_1305	6-phosphogluconate dehydrogenase, decarboxylating	COG0362	G	1.1.1.44	Cytoplasmic	1496719..1498173 [-]	8.41E-07	**-1.55**
uvrD	APL_1326	DNA helicase II	COG0210	L	3.6.1.-	Cytoplasmic	1519784..1521982 [+]	1.95E-04	**-1.54**
ape0378	APL_0357	hypothetical protein	COG3381	R			399573..400142 [-]	1.02E-08	**-1.54**
ape0189	APL_0180	hypothetical protein				CytoplasmicMembrane	199236..200000 [-]	4.99E-04	**-1.54**
ape2153	APL_2016	Ferrioxamine B receptor precursor	COG1629	P		OuterMembrane	2242636..2244489 [+]	2.70E-03	**-1.54**
ape0961	APL_0890	hypothetical protein	COG0679	R		CytoplasmicMembrane	1031364..1032296 [-]	3.02E-04	**-1.54**
frdA	APL_1529	fumarate reductase flavoprotein subunit	COG1053	C	1.3.99.1	Periplasmic	1742294..1744093 [-]	6.55E-03	**-1.53**
ape0921	APL_0852	putative FAD flavoprotein oxidase	COG1053	C			988874..990472 [+]	1.17E-09	**-1.53**
lamB1	APL_1235	maltoporin-1 precursor	COG4580	G		OuterMembrane	1418968..1420242 [-]	2.50E-05	**-1.53**
ape2024	APL_1894	hypothetical protein					2126121..2126351 [-]	7.02E-03	**-1.53**
ape1027	APL_0947	transposase					1087883..1089004 [-]	3.77E-05	**-1.53**
pgm	APL_0591	phosphoglucomutase/phosphomannomutase	COG1109	G	5.4.2.8		673326..674984 [+]	3.68E-05	**-1.53**
slyB	APL_0037	outer membrane lipoprotein	COG3133	M		CytoplasmicMembrane	41169..41633 [-]	2.13E-03	**-1.53**
artM	APL_1351	arginine transport system permease protein ArtM	COG4160	E		CytoplasmicMembrane	1549925..1550608 [-]	2.66E-07	**-1.53**

ape1118	APL_1031	hypothetical protein					1195472..1196212 [+]	4.76E-07	**-1.53**
ape1340	APL_1242	putative deoxyribonuclease	COG0084	L	3.1.21.-		1429516..1430310 [+]	6.62E-04	**-1.52**
galE	APL_1301	UDP-glucose 4-epimerase	COG1087	M	5.1.3.2	Cytoplasmic	1491032..1492048 [-]	3.21E-08	**-1.52**
ape0011	APL_0009	hypothetical protein					9914..10372 [+]	5.28E-05	**-1.52**
menD	APL_1750	Menaquinone biosynthesis protein menD	COG1165	H	2.5.1.64		1986901..1988607 [-]	8.60E-06	**-1.52**
sapD	APL_0793	peptide transport system ATP-binding protein SapD	COG4170	V		CytoplasmicMembrane	914274..915311 [-]	2.20E-05	**-1.52**
lbgB	APL_0979	putative D-glycero-D-manno-heptosyl transferase	COG0859	M			1132654..1133694 [-]	2.01E-07	**-1.52**
asnA	APL_1837	aspartate-ammonia ligase	COG2502	E	6.3.1.1	Cytoplasmic	2061014..2062006 [-]	1.60E-03	**-1.52**
fdhD	APL_0891	formate dehydrogenase accessory protein-like	COG1526	C		Cytoplasmic	1032298..1033104 [-]	1.83E-04	**-1.52**
proC	APL_0160	pyrroline-5-carboxylate reductase	COG0345	E	1.5.1.2		178027..178857 [-]	4.39E-06	**-1.52**
bioF	APL_0941	8-amino-7-oxononanoate synthase	COG0156	H	2.3.1.47		1081651..1082814 [-]	1.90E-03	**-1.52**
nusG	APL_1717	Transcription antitermination protein nusG	COG0250	K		Cytoplasmic	1948786..1949316 [+]	2.53E-03	**-1.52**
sppA	APL_1268	protease 4	COG0616	OU	3.4.21.-		1456773..1458629 [+]	3.88E-05	**-1.51**
gltS	APL_0967	sodium/glutamate symport carrier protein	COG0786	E		CytoplasmicMembrane	1120735..1121952 [+]	5.08E-05	**-1.51**
wecE	APL_1549	TDP-4-keto-6-deoxy-D-glucose transaminase	COG0399	M			1771474..1772619 [-]	2.29E-04	**-1.51**
ape0237	APL_0226	hypothetical protein					247033..247569 [+]	3.99E-03	**-1.51**
bcp	APL_0846	putative peroxiredoxin Bcp	COG1225	O			981448..981918 [-]	1.38E-05	**-1.51**
rsmC	APL_2005	Ribosomal RNA small subunit methyltransferase C	COG2813	J	2.1.1.52	Cytoplasmic	2230377..2231366 [-]	4.06E-06	**-1.51**
rph	APL_0055	ribonuclease PH	COG0689	J	2.7.7.56	Cytoplasmic	61124..61840 [-]	2.18E-04	**-1.51**
fdnI	APL_0895	formate dehydrogenase, cytochrome b556 subunit	COG2864	C	1.2.1.2	CytoplasmicMembrane	1037474..1038145 [+]	3.42E-03	**-1.51**
mqo	APL_1414	putative malate:quinone oxidoreductase	COG0579	R	1.1.99.16		1616829..1618304 [-]	2.12E-08	**-1.51**
fadR	APL_0323	fatty acid metabolism regulator protein	COG2186	K		Cytoplasmic	360456..361184 [+]	7.01E-07	**-1.50**
ape1257	APL_1166	putative ubiquinone/menaquinone biosynthesis methyltransferase	COG0500	QR			1343503..1344258 [+]	1.68E-10	**-1.50**
purK	APL_0661	phosphoribosylaminoimidazole carboxylase ATPase subunit	COG0026	F	4.1.1.21	Cytoplasmic	754466..755554 [+]	4.00E-05	**-1.50**
purT	APL_1106	putative phosphoribosylglycinamide formyltransferase 2	COG0027	F	2.1.2.-	Cytoplasmic	1281666..1282847 [-]	1.27E-03	**-1.50**
proA	APL_1951	Gamma-glutamyl phosphate reductase	COG0014	E	1.2.1.41	Cytoplasmic	2181067..2182305 [-]	9.78E-05	**-1.50**
ape1392	APL_1291	hypothetical protein	COG0767	Q		CytoplasmicMembrane	1480329..1481033 [-]	3.01E-06	**-1.50**
ape1471	APL_1366	hypothetical protein	COG4623	M			1562283..1563650 [-]	1.93E-06	**-1.50**
ape0612	APL_0561	hypothetical protein					629789..630211 [+]	6.33E-03	**-1.50**

G 5 Preparative gel electrophoresis for protein identification by mass spectrometry



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71
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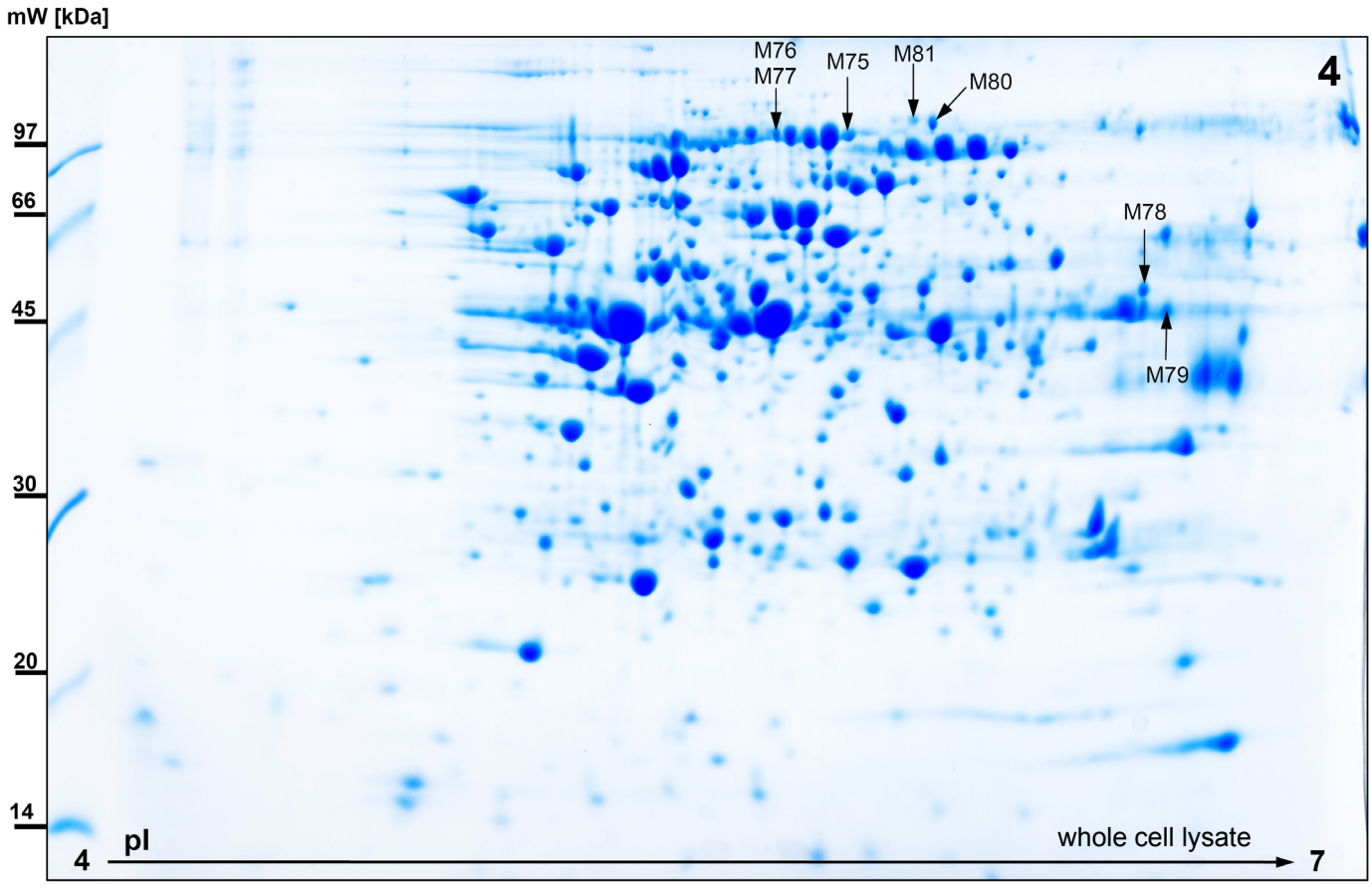
4
pI
whole cell lysate
7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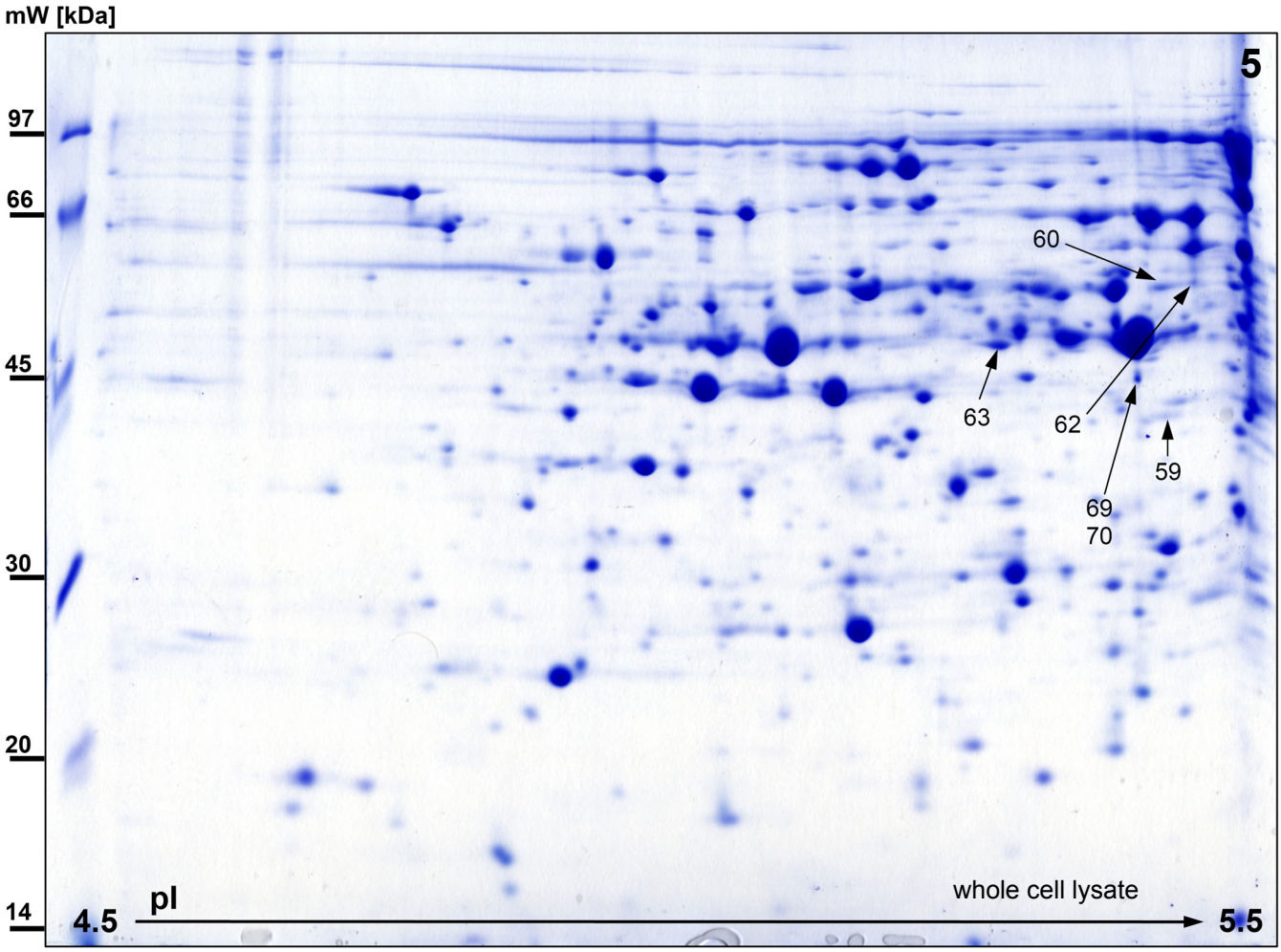
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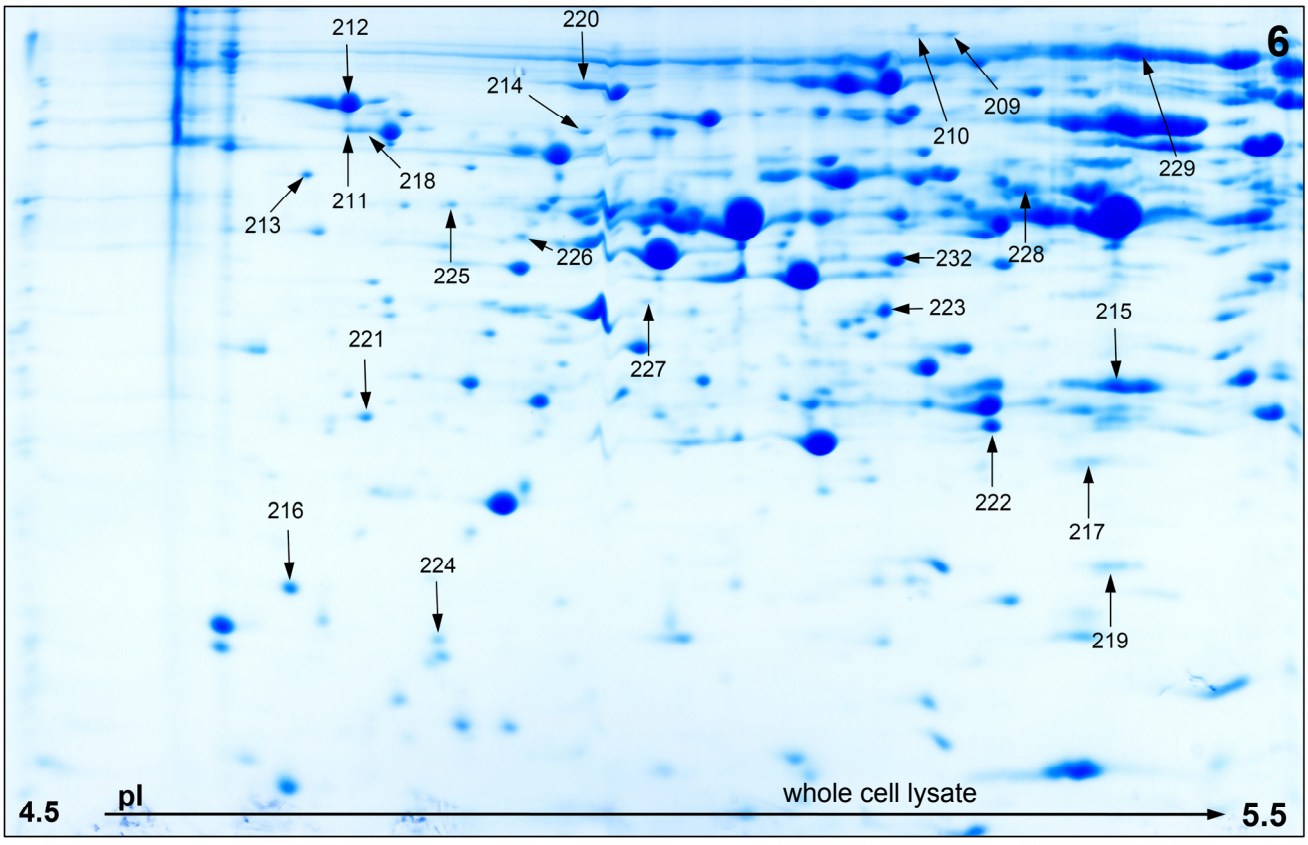
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pI
whole cell lysate
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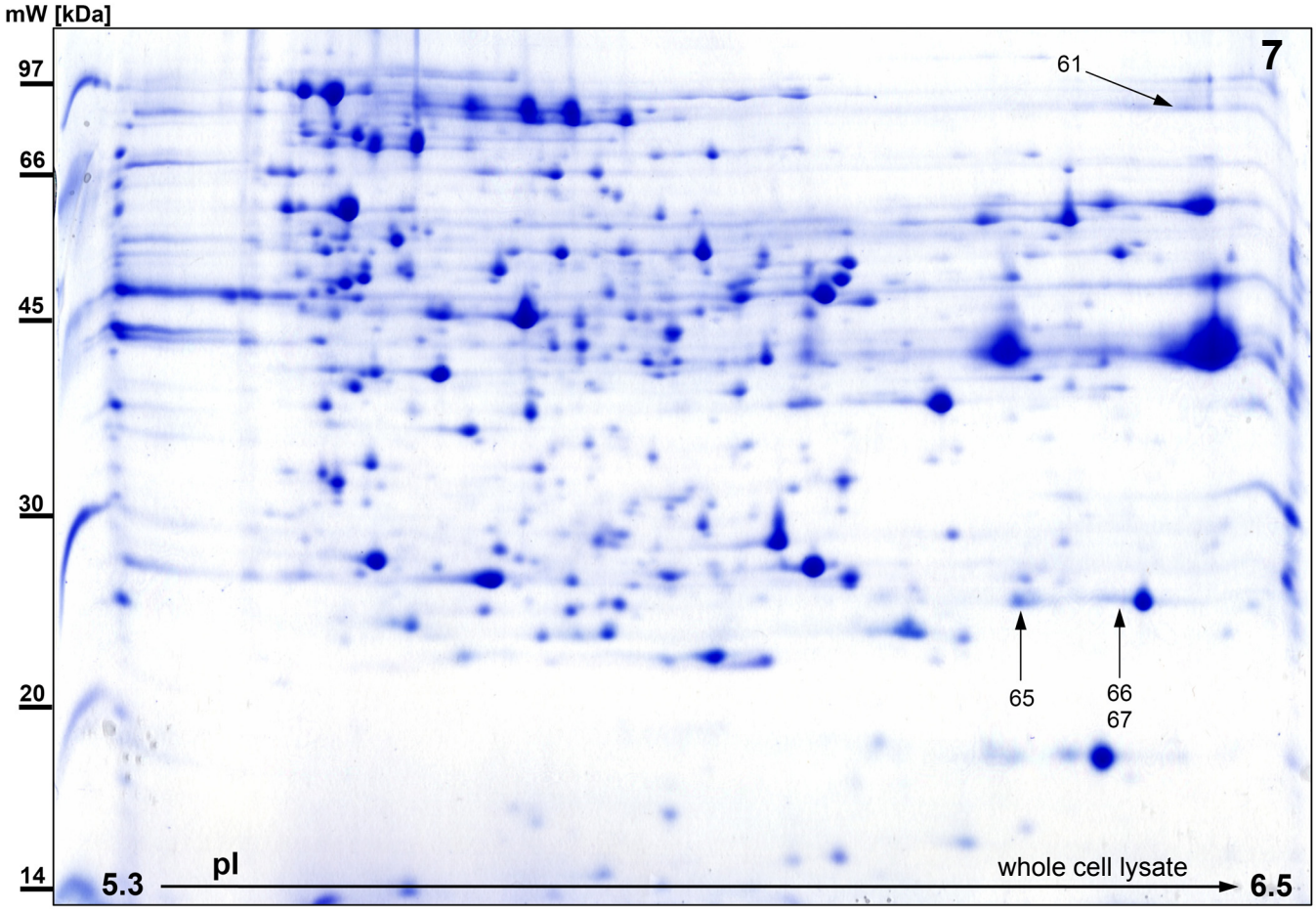
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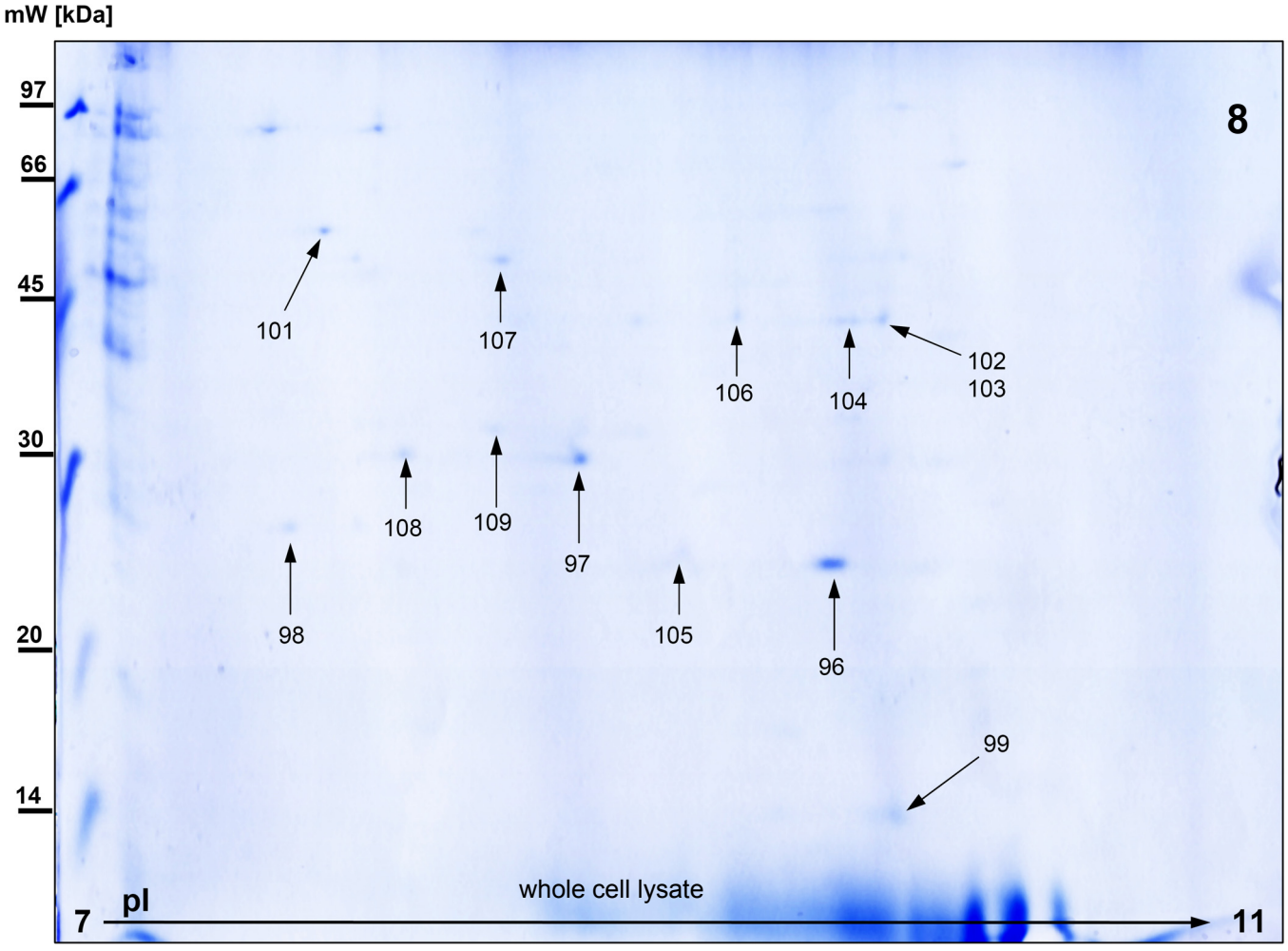
6.5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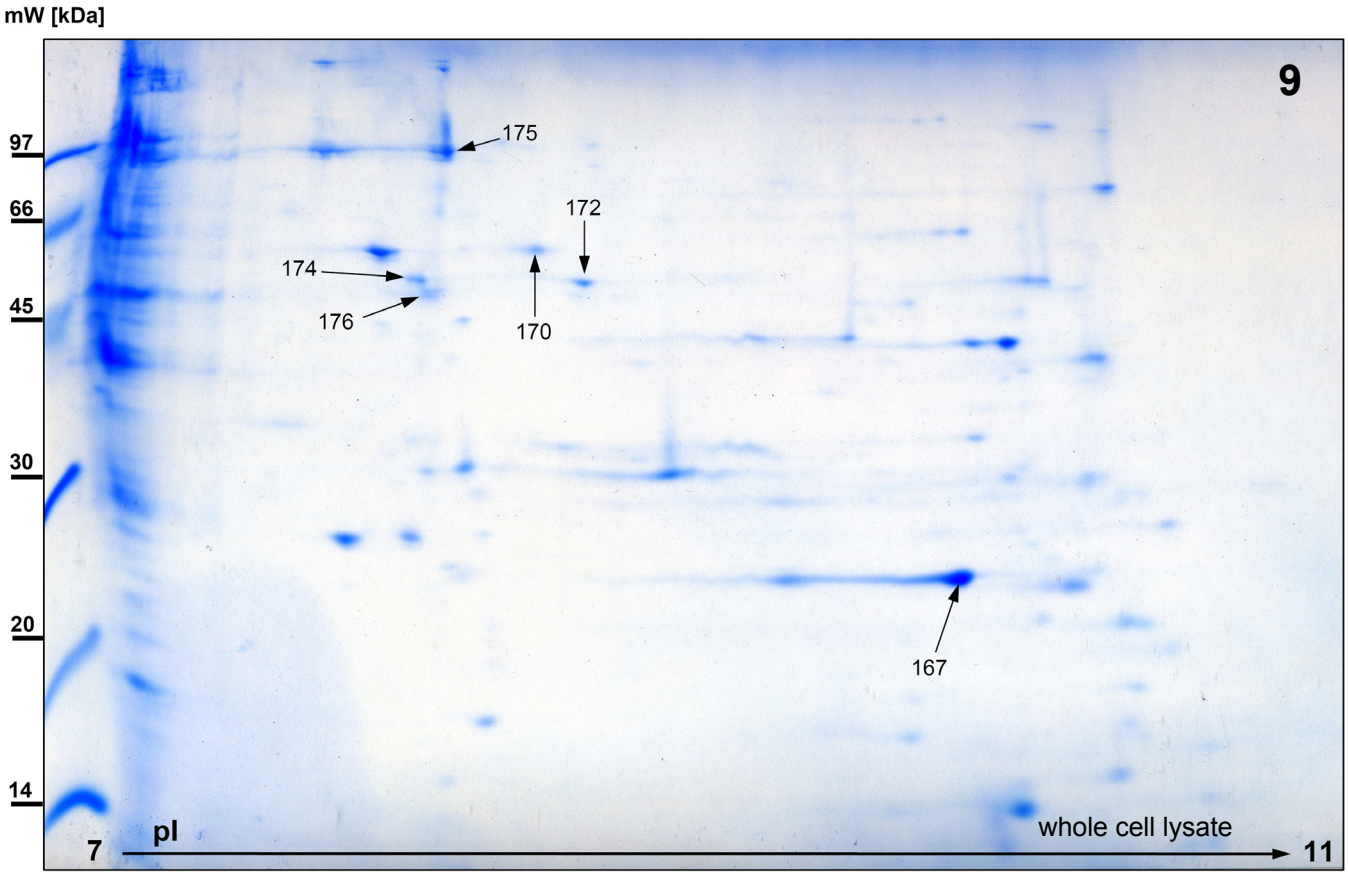
11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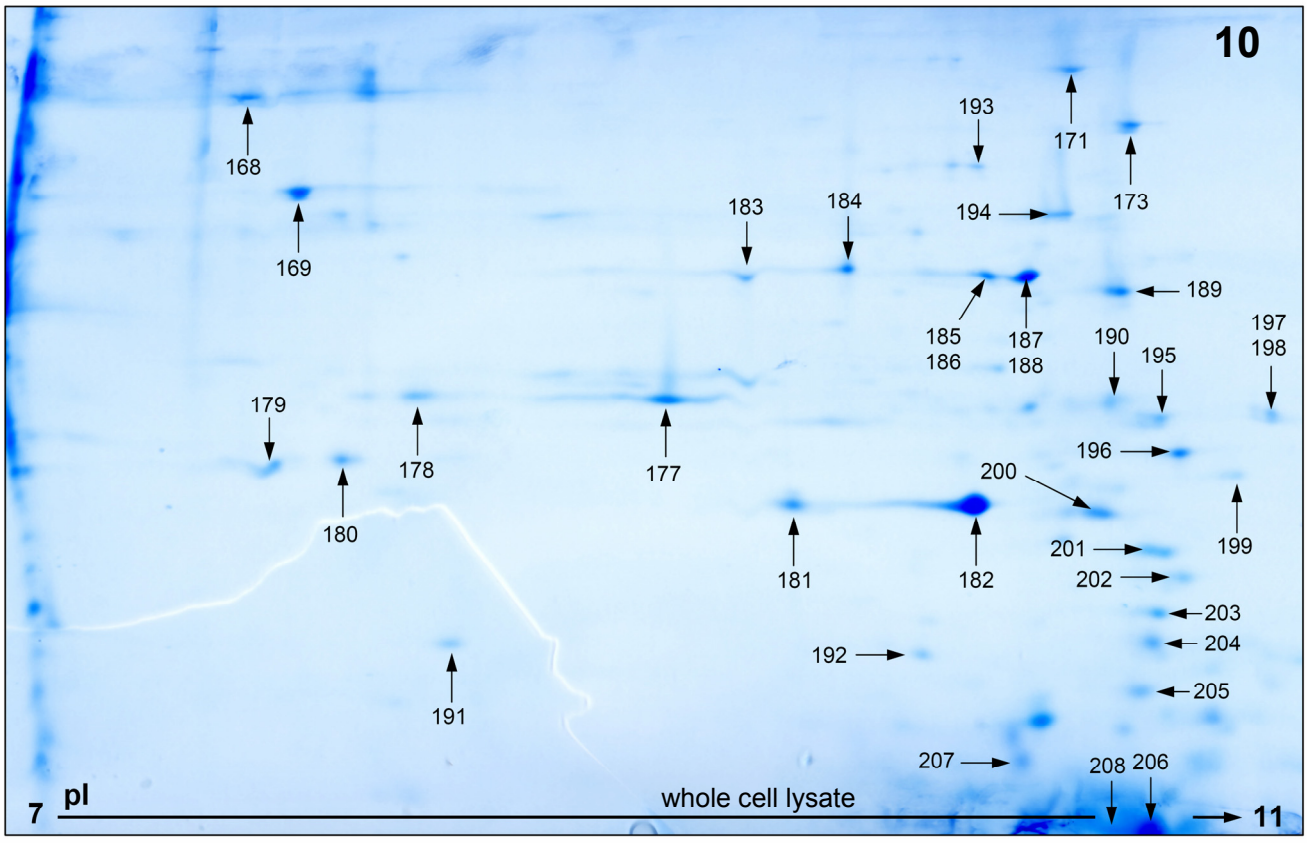
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pI
whole cell lysate
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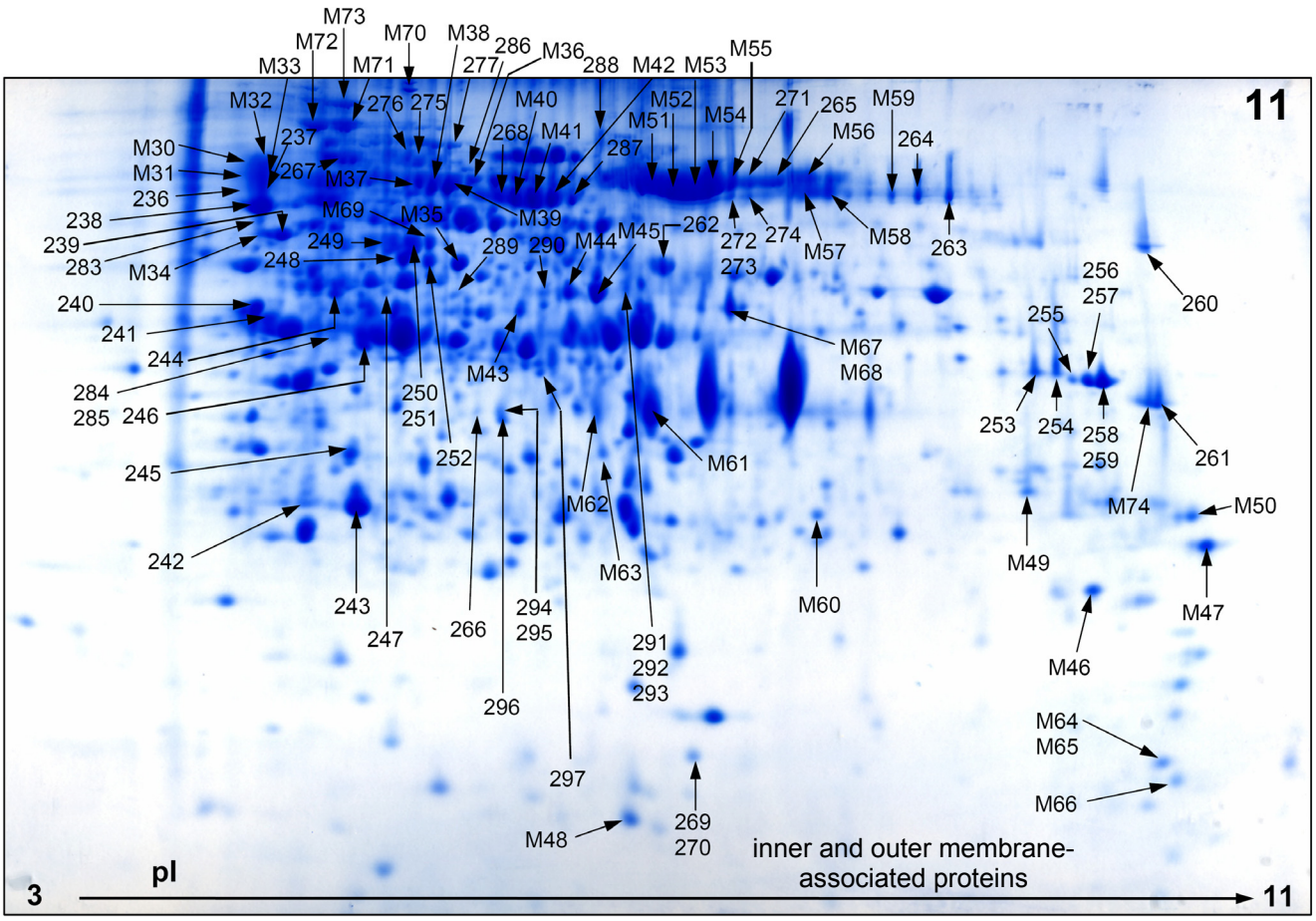
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M66
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M48
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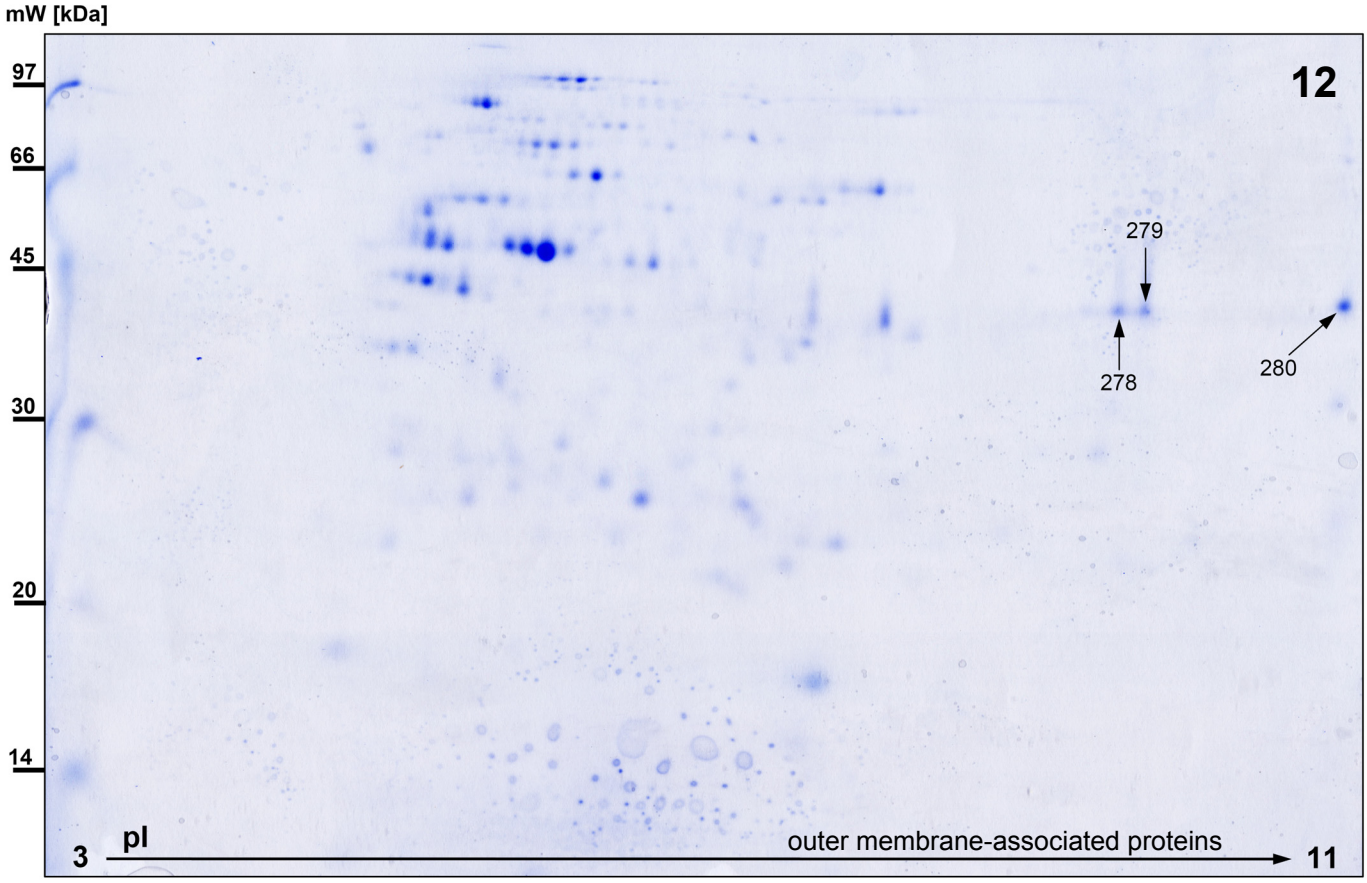
inner and outer membrane-
associated proteins
3
pI
11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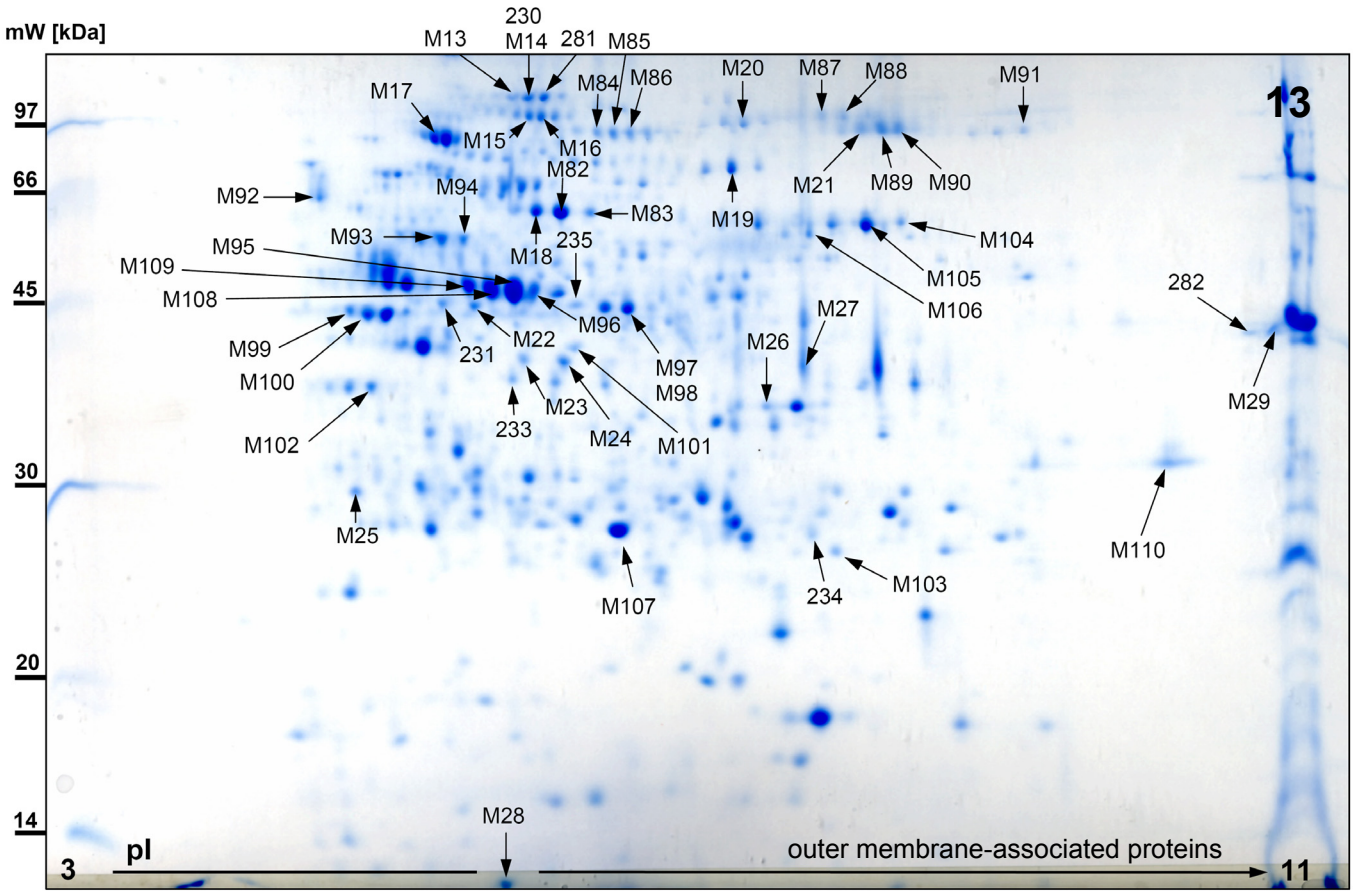
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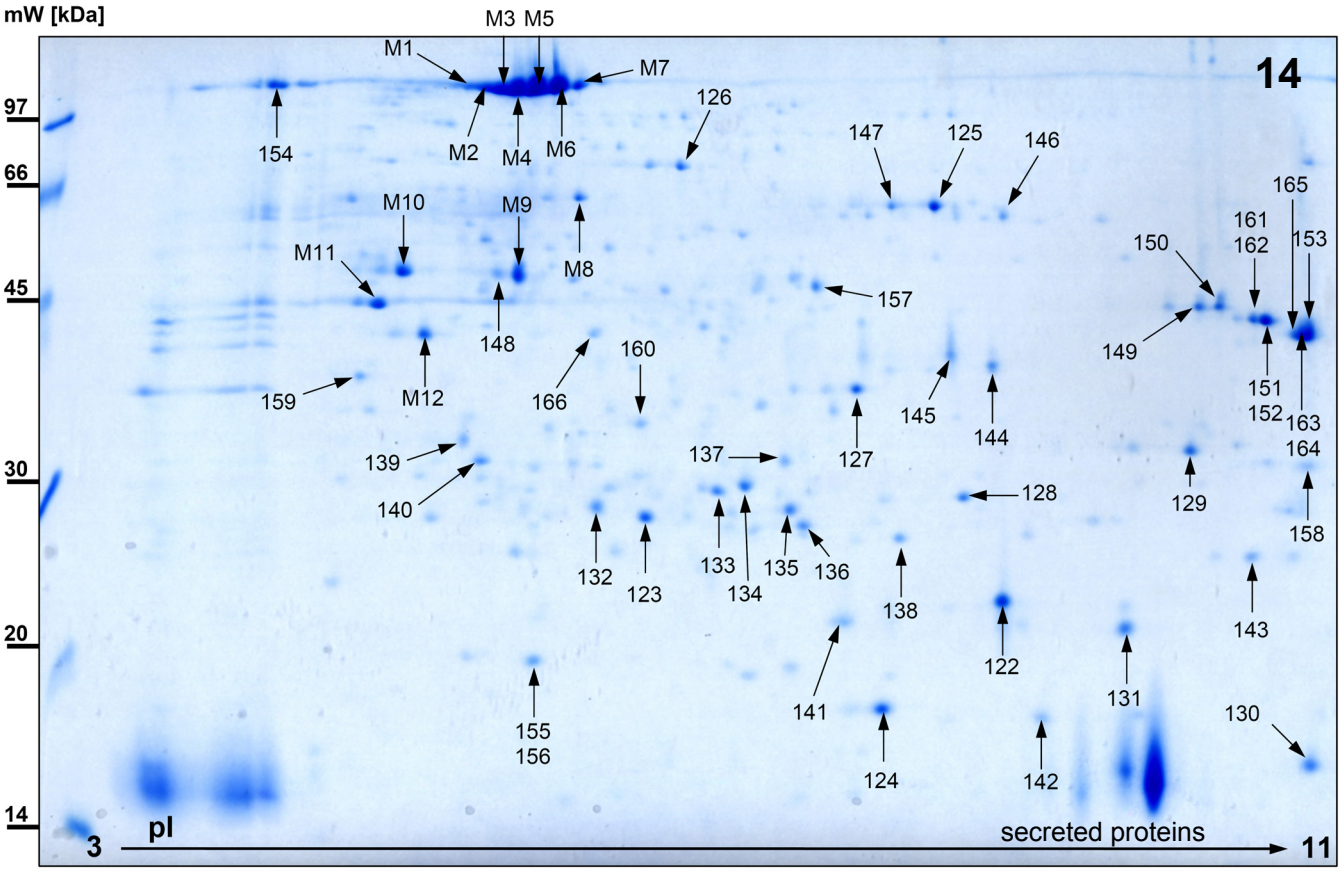
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secreted proteins
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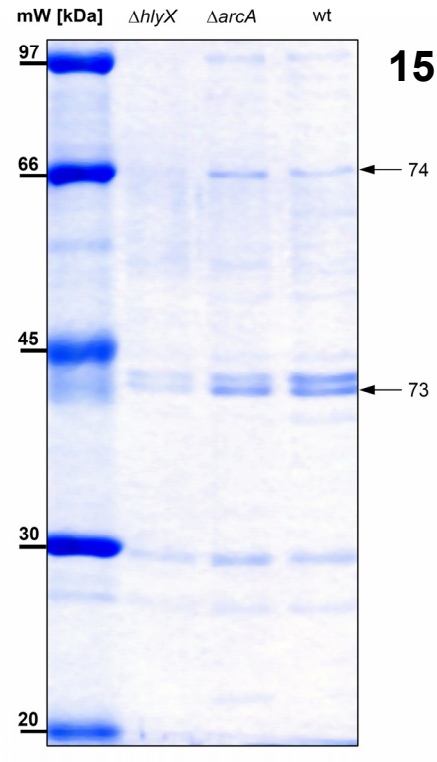
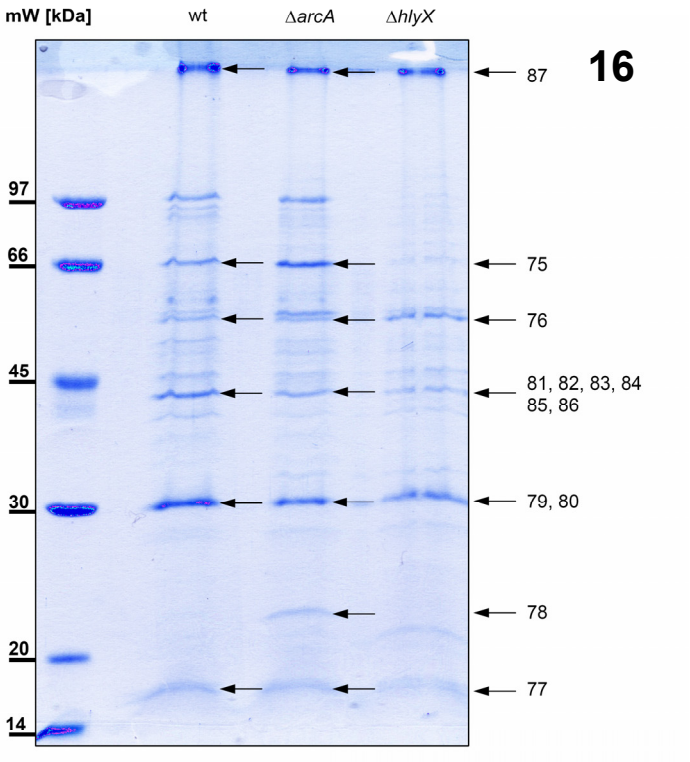
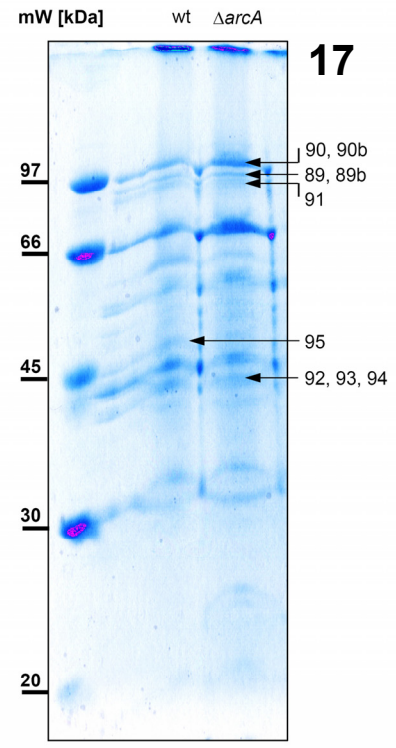


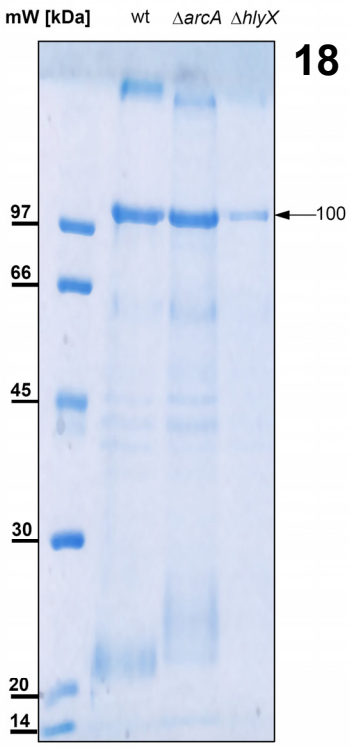
outer membrane proteins



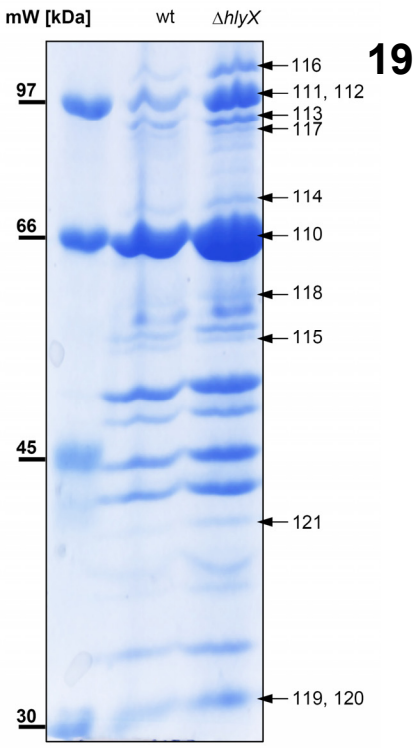
outer membrane proteins



outer membrane proteins



secreted proteins



outer membrane proteins

Overview preparative gels. Proteins of interest were excised from Coomassie stained gels and analysed by mass spectrometry.

gel #	first dimension			acryl-amide	AP76 strain and amount of protein	growth	protein preparation	total protein	staining
	pI	gradient	length						
1	3 to 10	linear	24 cm	12.5 %	600 µg Δ*arcA*	anaerobic	whole cell lysate	600 µg	colloidal Coomassie
2	4 to 7	linear	24 cm	12.5 %	600 µg Δ*arcA*	anaerobic	whole cell lysate	600 µg	colloidal Coomassie
3	4 to 7	linear	24 cm	12.5 %	67 µg wt + 67 µg Δ*arcA* + 17 µg Δ*hlyX*	anaerobic	whole cell lysate	150 µg	Cy2, Cy3, Cy5; poststaining with colloidal Coomassie
4	4 to 7	linear	24 cm	12.5 %	750 µg wt, 750 µg Δ*arcA*	anaerobic	whole cell lysate	1500 µg	colloidal Coomassie
5	4.5 to 5.5	linear	24 cm	12.5 %	600 µg wt, 600 µg Δ*arcA*, 300 µg Δ*hlyX*	anaerobic	whole cell lysate	1500 µg	colloidal Coomassie
6	4.5 to 5.5	linear	24 cm	12.5 %	667 µg wt, 667µg Δ*arcA*, 667µg Δ*hlyX*	anaerobic	whole cell lysate	2000 µg	colloidal Coomassie
7	5.3 to 6.5	linear	24 cm	12.5 %	600 µg wt, 600 µg Δ*arcA*, 300 µg Δ*hlyX*	anaerobic	whole cell lysate	1500 µg	colloidal Coomassie
8	7 to 11	non linear	24 cm	12.5 %	167 µg wt, 167 µg Δ*arcA*, 42 µg Δ*hlyX*	anaerobic	whole cell lysate	375 µg	Cy2, Cy3, Cy5; poststaining with colloidal Coomassie
9	7 to 11	non linear	24 cm	12.5 %	750 µg wt, 750 µg Δ*arcA*	anaerobic	whole cell lysate	1500 µg	colloidal Coomassie
10	7 to 11	non linear	24 cm	12.5 %	667 µg wt, 667µg Δ*arcA*, 667µg Δ*hlyX*	anaerobic	whole cell lysate	2000 µg	colloidal Coomassie
11	3 to 11	non linear	24 cm	12.5 %	wt	anaerobic	inner and outer membrane-associated proteins	nd	colloidal Coomassie
12	3 to 11	non linear	24 cm	12.5 %	67 µg wt + 67 µg Δ*hlyX* + 17 µg Δ*arcA*	anaerobic	outer membrane-associated proteins	150 µg	Cy2, Cy3, Cy5; poststaining with colloidal Coomassie
13	3 to 11	non linear	24 cm	12.5 %	400 µg Δ*arcA*	anaerobic	outer membrane-associated proteins	400 µg	colloidal Coomassie
14	3 to 11	non linear	24 cm	12.5 %	wt, Δ*arcA*, Δ*hlyX*	anaerobic	secreted proteins	nd	colloidal Coomassie
15	one dimensional			10.8%	a: Δ*hlyX*; b: Δ*arcA*; c: wt	anaerobic	outer membranes	nd	Coomassie
16	one dimensional			10.8%	a: wt; b: Δ*arcA*; c: Δ*hlyX*	anaerobic	outer membranes	nd	Coomassie
17	one dimensional			10.8%	a: wt; b: Δ*arcA*	anaerobic	outer membranes	nd	Coomassie
18	one dimensional			10.8%	a: wt; b: Δ*arcA*; c: Δ*hlyX*	anaerobic	secreted proteins	nd	Coomassie
19	one dimensional			10.8%	a: wt; b: Δ*hlyX*	aerobic	outer membranes	nd	colloidal Coomassie

nd: not determined

G 6 Mass Spectrometry

G 6.1 Q-TOF MSMS

Results of Q-TOF tandem mass spectrometry

MS # [a]	gel # [b]	accesion #	proba-bility [%] [c]	matched peptides [d]	cover-age [%] [e]	mW [Da]	pI	average mass error [ppm] [f]	protein [g]
1	1	126207970	100	7	22.75	44901.09	5.21	19.127	NADP dependent malic enzyme NADP ME
2	1	126208914	100	7	25.45	36913.55	5.55	5.568	fructose 1 6 bisphosphatase
3	1	126207928	100	5	25.43	25535.22	5.42	53.739	hypothetical protein APL 0444
4	1	126207930	100	5	22.82	26335.58	7.91	36.807	putative dehydrogenase subunit
5	2	126207739	100	2	14.09	23741.85	6.71	22.496	manganese superoxide dismutase
6	2	126208488	100	7	37.08	25998.07	5.12	8.456	purine nucleoside phosphorylase DeoD like protein
7	2	126209301	100	6	34.25	27258.83	6.32	7.283	uridine phosphorylase
8	2	126208914	100	5	17.07	36913.55	5.55	35.397	fructose 1 6 bisphosphatase
9	2	126208308	100	4	21.59	25889.46	6.35	12.395	2 3 bisphosphoglycerate dependent phosphoglycerate mutase
10	2	126209088	100	1	5.70	25572.24	7.09	4.261	putative periplasmic binding protein CbiK
11	2	126209359	100	2	11.43	19453.88	4.95	7.567	inorganic pyrophosphatase
12	2	126209413	100	5	18.05	36743.72	6.02	8.922	alcohol dehydrogenase 1
13	2	126208249	100	2	6.33	50520.37	6.11	8.572	dihydrolipoyl dehydrogenase
14	3	126208251	100	7	8.48	98856.58	5.57	70.921	pyruvate dehydrogenase E1 component
15	3	126208510	100	8	14.68	86119.01	5.89	36.074	formate acetyltransferase
16	3	126208251	100	3	5.09	98856.58	5.57	52.572	pyruvate dehydrogenase E1 component
17	3	126208510	100	10	16.75	86119.01	5.89	14.5	formate acetyltransferase
18	3	126208510	100	6	8.44	86119.01	5.89	43.692	formate acetyltransferase
19	3	126208251	100	4	6.11	98856.58	5.57	36.589	pyruvate dehydrogenase E1 component
20	3	126208348	100	3	5.34	97687.67	5.45	29.496	leucyl tRNA synthetase
21	3	126208510	100	2	3.90	86119.01	5.89	40.661	formate acetyltransferase
22	3	126208510	100	8	14.16	86119.01	5.89	13.676	formate acetyltransferase
23	3	126208250	100	6	9.34	66103.72	5.08	7.837	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2
24	3	126208059	100	11	17.76	77349.28	5.00	23.59	polyribonucleotide nucleotidyltransferase
25	3	126208091	100	9	17.74	86309.02	5.18	7.268	phenylalanyl tRNA synthetase beta chain
26	3	126208091	100	4	7.42	86309.02	5.18	9.936	phenylalanyl tRNA synthetase beta chain
27	3	126208787	100	8	15.01	62702.77	4.81	16.392	phosphoenolpyruvate protein phosphotransferase
28	3	126208485	0	1	1.26	95276.65	6.95	51.661	aldehyde alcohol dehydrogenase 2
29	3	126208073	100	6	14.67	59738.73	5.16	37.014	phosphoglucomutase phosphomannomutase
30	3	126208251	100	9	10.75	98856.58	5.57	45.976	pyruvate dehydrogenase E1 component
31	3	126208250	100	2	3.48	66103.72	5.08	84.076	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2
32	3	126208439	100	1	5.73	22137.64	9.11	15.964	hypothetical protein APL 0965
33	3	126208276	100	13	34.33	59390.11	5.41	10.339	phosphoenolpyruvate carboxykinase ATP
34	3	126208759	100	4	12.28	49169.02	6.37	3.829	probable periplasmic serine protease do hhoA like precursor
35	3	126208344	100	3	6.67	48334.86	5.70	6.893	seryl tRNA synthetase
36	3	126208127	100	9	33.58	43555.48	5.98	3.542	acetate kinase
37	3	126207872	100	1	2.09	46371.36	5.65	4.362	peptidase B
38	3	126208457	100	12	23.80	73216.57	5.72	36.494	transketolase 2
39	3	126208249	100	2	5.70	50520.37	6.11	43.72	dihydrolipoyl dehydrogenase
40	3	126209465	100	3	7.51	53794.55	5.62	44.068	putative aldehyde dehydrogenase aldA
41	3	126208168	100	4	5.95	91194.05	7.12	10.807	trimethylamine N oxide reductase precursor
42	3	126208249	100	1	3.80	50520.37	6.11	5.877	dihydrolipoyl dehydrogenase
43	3	126208607	100	6	13.74	60376.76	6.62	4.406	glucose 6 phosphate isomerase
44	3	126208276	100	11	29.29	59390.11	5.41	45.039	phosphoenolpyruvate carboxykinase ATP
45	3	126208439	100	1	5.73	22137.64	9.11	20.005	hypothetical protein APL 0965
46	3	126208457	100	9	17.07	73216.57	5.72	59.553	transketolase 2

47	3	126208607	100	11	28.57	60376.76	6.62	8.496	glucose 6 phosphate isomerase
48	3	126209095	100	4	10.98	67158.64	5.54	9.517	glucosamine fructose 6 phosphate aminotransferase isomerizing
49	3	126209095	100	8	19.51	67158.64	5.54	6.71	glucosamine fructose 6 phosphate aminotransferase isomerizing
50	3	126209095	100	12	30.82	67158.64	5.54	8.791	glucosamine fructose 6 phosphate aminotransferase isomerizing
51	3	126209095	100	7	19.02	67158.64	5.54	12.358	glucosamine fructose 6 phosphate aminotransferase isomerizing
52	3	126208741	100	3	8.56	54156.15	5.94	5.604	glutamyl tRNA synthetase
53	3	126208127	100	11	42.29	43555.48	5.98	7.61	acetate kinase
54	3	126207742	100	6	19.01	52903.67	5.62	4.529	aminoacyl histidine dipeptidase
55	3	126208563	100	7	20.00	51649.58	5.29	6.977	aspartate ammonia lyase
56	3	126209095	100	2	4.43	67158.64	5.54	18.73	glucosamine fructose 6 phosphate aminotransferase isomerizing
57	2	126208762	100	2	10.10	33325.92	5.93	8.562	malate dehydrogenase
58	2	126207851	100	3	18.69	21826.06	4.64	45.273	heat shock protein HSP 70 cofactor
59	5	126208914	100	3	10.18	36913.55	5.55	7.834	fructose 1 6 bisphosphatase
60	5	126209465	100	2	6.09	53794.55	5.62	35.278	putative aldehyde dehydrogenase aldA
61	7	126208168	100	2	2.79	91194.05	7.12	40.527	trimethylamine N oxide reductase precursor
62	5	126208563	100	3	6.74	51649.58	5.29	11.282	aspartate ammonia lyase
63	5	126207970	100	5	12.32	44901.09	5.21	8.24	NADP dependent malic enzyme NADP ME
64	3	126208168	100	1	1.46	91194.05	7.12	21.599	trimethylamine N oxide reductase precursor
65	7	126207739	100	4	24.88	23741.85	6.71	10.021	manganese superoxide dismutase
66	7	126207739	100	2	13.62	23741.85	6.71	50.387	manganese superoxide dismutase
67	7	126207739	100	1	4.70	23741.85	6.71	5.422	manganese superoxide dismutase
68	3	126208594	100	2	10.49	35492.30	6.46	17.785	6 phosphofructokinase
69	5	126207803	100	4	10.50	40820.23	5.42	6.62	hypothetical protein APL 0319
70	5	126207505	100	1	2.04	44072.73	9.40	52.877	cell division protein FtsW
71	3	126207550	100	6	20.00	34772.19	5.21	19.148	transaldolase
72	3	126208946	100	4	18.44	26683.22	5.25	41.718	hybrid peroxiredoxin HyPrx5
73	15	126208129	100	3	7.26	39570.96	9.13	6.136	outer membrane protein P2 precursor OMP P2
74	15	126207764	100	2	3.96	73081.29	9.78	11.869	iron regulated outer membrane protein B
75	16	126207764	100	5	10.98	73081.29	9.78	18.404	iron regulated outer membrane protein B
76	16	126209399	100	5	18.68	52290.10	5.20	15.391	glutamine synthetase
77	16	126208542	100	1	6.67	19000.47	4.89	12.929	ferritin like protein 2
78	16	126208558	100	3	28.37	22923.01	8.48	4.701	outer membrane protein W precursor
79	16	126208885	100	7	16.03	39507.21	8.92	10.003	outer membrane protein P5 precursor
80	16	126209314	100	5	11.54	38708.01	9.72	15.591	outer membrane protein P5 precursor OMP P5
81	16	126208129	100	1	4.75	39570.96	9.13	7.043	outer membrane protein P2 precursor OMP P2
82	16	126209314	51	1	2.75	38708.01	9.72	17.136	outer membrane protein P5 precursor OMP P5
83	16	126208885	50	1	2.72	39507.21	8.92	17.136	outer membrane protein P5 precursor
84	16	126208885	100	3	9.24	39507.21	8.92	24.627	outer membrane protein P5 precursor
85	16	126208129	100	1	2.51	39570.96	9.13	12.696	outer membrane protein P2 precursor OMP P2
86	16	126209314	0	1	3.30	38708.01	9.72	19.529	outer membrane protein P5 precursor OMP P5
87	16	126208129	100	1	4.75	39570.96	9.13	1.771	outer membrane protein P2 precursor OMP P2
88	ns	126208249	100	7	20.25	50520.37	6.11	11.673	dihydrolipoyl dehydrogenase
89	17	126207895	100	8	15.39	89087.97	6.63	12.135	protective surface antigen D15 precursor
89b	17	126208885	100	1	6.52	39507.21	8.92	7.059	outer membrane protein P5 precursor
90	17	126207848	100	7	8.05	103746.56	9.53	12.565	serotype specific antigen 1 precursor
90b	17	126208485	100	1	2.07	95276.65	6.95	22.966	aldehyde alcohol dehydrogenase 2
91	17	126209138	100	1	1.62	89674.50	7.60	18.27	anaerobic dimethyl sulfoxide reductase chain A precursor
92	17	126209314	100	7	26.92	38708.01	9.72	35.283	outer membrane protein P5 precursor OMP P5
93	17	126208885	100	1	6.52	39507.21	8.92	36.759	outer membrane protein P5 precursor
94	17	126208129	100	1	4.75	39570.96	9.13	39.725	outer membrane protein P2 precursor OMP P2
95	17	3927875	100	2	7.40	39683.28	6.78	5.01	outer membrane lipoprotein
96	8	126208755	100	5	18.87	23548.30	9.68	8.805	hypothetical protein APL 1289
97	8	126209294	100	2	10.69	36086.64	9.25	8.609	hypothetical protein APL 1832
98	8	126207750	100	8	48.41	27734.61	9.24	19.793	molybdate binding periplasmic protein precursor
99	8	126208189	100	2	31.53	13351.78	9.41	20.602	hypothetical protein APL 0709
100	18	126208432	100	16	28.98	102443.53	5.53	16.279	RTX II toxin determinant A

101	8	126208075	100	4	11.29	52016.22	8.14	21.127	inosine 5 monophosphate dehydrogenase
102	8	126208885	100	2	9.24	39507.21	8.92	34.211	outer membrane protein P5 precursor
103	8	126209314	0	1	2.75	38708.01	9.72	40.801	outer membrane protein P5 precursor OMP P5
104	8	126208885	100	1	6.52	39507.21	8.92	33.332	outer membrane protein P5 precursor
105	8	126208755	100	1	5.19	23548.30	9.68	5.195	hypothetical protein APL 1289
106	8	126208129	100	4	15.64	39570.96	9.13	12.77	outer membrane protein P2 precursor OMP P2
107	8	126207929	100	4	12.37	51994.79	8.17	21.82	putative electron transport protein
108	8	126209294	100	2	7.55	36086.64	9.25	3.92	hypothetical protein APL 1832
109	8	126208247	100	1	1.83	60857.33	7.05	10.201	UshA precursor
110	19	126207764	100	14	29.42	73081.29	9.78	10.161	iron regulated outer membrane protein B
111	19	126207848	100	3	4.61	103746.56	9.53	2.878	serotype specific antigen 1 precursor
112	19	126209031	100	2	3.32	106656.44	9.41	2.949	transferrin binding protein 1 Tbp1
113	19	126207895	100	9	18.03	89087.97	6.63	7.122	protective surface antigen D15 precursor
114	19	78883538	60	7	19.78	72694.38	6.80	7.228	outer membrane ferric hydroxamate receptor FhuA
115	19	126209399	100	4	11.89	52290.10	5.20	10.886	glutamine synthetase
116	19	126208521	100	13	17.93	107589.00	8.92	33.327	hemoglobin binding protein A precursor
117	19	126208436	100	3	5.66	89767.17	7.99	1.288	organic solvent tolerance protein precursor
118	19	126207764	100	12	27.29	73081.29	9.78	8.993	iron regulated outer membrane protein B
119	19	126208885	100	2	10.33	39507.21	8.92	9.057	outer membrane protein P5 precursor
120	19	126209314	100	1	3.85	38708.01	9.72	2.176	outer membrane protein P5 precursor OMP P5
121	19	126209314	100	3	13.19	38708.01	9.72	3.567	outer membrane protein P5 precursor OMP P5
122	14	126208558	100	3	18.61	22923.01	8.48	16.746	outer membrane protein W precursor
123	14	126209104	100	4	20.58	26478.89	7.33	35.563	putative FKBP type peptidyl prolyl cis trans isomerase
124	14	126207492	100	1	6.32	20194.19	6.75	12.192	superoxide dismutase Cu Zn precursor
125	14	126208247	100	6	11.70	60857.33	7.05	17.572	UshA precursor
126	14	126208128	100	2	3.19	72535.22	6.36	34.881	2 3 cyclic nucleotide 2 phosphodiesterase precursor
127	14	126208904	100	3	10.15	35710.29	6.50	7.103	high affinity zinc uptake system protein ZnuA precursor
128	14	126208194	100	4	14.57	32490.47	8.51	4.759	ABC transport system periplasmic protein
129	14	126209294	100	1	4.40	36086.64	9.25	5.061	hypothetical protein APL 1832
130	14	126207537	100	3	20.35	18203.46	9.87	6.63	hypothetical protein APL 0049
131	14	3927875	100	3	10.41	39683.28	6.78	8.458	outer membrane lipoprotein
132	14	126209385	100	3	12.89	26505.78	5.58	9.353	Triosephosphate isomerase
133	14	126209136	100	1	4.83	30115.26	8.49	0.982	D ribose binding periplasmic protein precursor
134	14	126207760	100	4	19.46	32849.36	8.34	5.258	iron chelated ABC transporter periplasmic binding protein
135	14	126208308	100	4	23.79	25889.46	6.35	7.905	2 3 bisphosphoglycerate dependent phosphoglycerate mutase
136	14	126209088	100	4	19.74	25572.24	7.09	10.437	putative periplasmic binding protein CbiK
137	14	126209052	100	2	8.86	34474.57	6.81	10.178	predicted TRAP transporter solute receptor
138	14	126207739	100	1	6.10	23741.85	6.71	17.986	manganese superoxide dismutase
139	14	126208049	100	3	15.19	30222.63	5.26	17.594	elongation factor Ts
140	14	126208884	100	3	11.52	35417.66	5.77	7.038	D galactose binding periplasmic protein precursor
141	14	126209330	100	2	8.96	23473.09	8.67	2.747	thiol disulfide interchange protein dsbA precursor
142	14	126208572	100	3	18.92	21892.33	8.60	17.973	hypothetical protein APL 1100
143	14	126208432	100	1	1.05	102443.53	5.53	62.935	RTX II toxin determinant A
144	14	126208910	100	2	5.49	37835.43	8.07	12.602	ABC type Fe3 transport system periplasmic component
145	14	126207918	100	3	11.38	35691.35	6.99	17.091	glyceraldehyde 3 phosphate dehydrogenase
146	14	126207552	100	2	4.50	59965.18	8.03	20.193	periplasmic dipeptide transport protein
147	14	126208247	100	2	4.94	60857.33	7.05	20.816	UshA precursor
148	14	126208862	50	1	3.30	43439.30	5.40	28.329	elongation factor Tu
149	14	126208129	100	2	5.03	39570.96	9.13	17.547	outer membrane protein P2 precursor OMP P2
150	14	126208129	100	4	8.10	39570.96	9.13	10.827	outer membrane protein P2 precursor OMP P2
151	14	126208885	100	6	18.21	39507.21	8.92	11.955	outer membrane protein P5 precursor
152	14	126209314	0	2	3.30	38708.01	9.72	12.785	outer membrane protein P5 precursor OMP P5
153	14	126209314	100	6	18.13	38708.01	9.72	13.131	outer membrane protein P5 precursor OMP P5
154	14	126208432	100	6	7.22	102443.53	5.53	30.916	RTX II toxin determinant A
155	14	126208958	100	1	4.76	20732.61	5.71	2.492	fine tangled pili major subunit
156	14	75429133	34	1	4.79	20960.85	5.90	2.492	starvation inducible DNA binding protein Actinobacillus succinogenes

157	14	126207911	100	5	12.78	48462.46	6.65	12.104	NADP specific glutamate dehydrogenase
158	14	126209314	100	1	3.57	38708.01	9.72	24.883	outer membrane protein P5 precursor OMP P5
159	14	126207550	100	3	11.11	34772.19	5.21	36.949	transaldolase
160	14	126208762	100	6	24.29	33325.92	5.93	42.702	malate dehydrogenase
161	14	126208885	100	4	15.22	39507.21	8.92	7.518	outer membrane protein P5 precursor
162	14	126209314	0	1	2.75	38708.01	9.72	8.033	outer membrane protein P5 precursor OMP P5
163	14	126209314	100	6	18.96	38708.01	9.72	21.522	outer membrane protein P5 precursor OMP P5
164	14	126208885	0	1	3.26	39507.21	8.92	22.493	outer membrane protein P5 precursor
165	14	126209314	100	4	11.81	38708.01	9.72	6.385	outer membrane protein P5 precursor OMP P5
166	14	126207493	100	2	7.03	40395.02	5.60	23.551	aspartate semialdehyde dehydrogenase Asd
167	9	126208755	100	5	19.81	23548.30	9.68	18.001	hypothetical protein APL 1289
168	10	126208893	100	3	3.99	93511.73	8.21	29.727	periplasmic nitrate reductase precursor
169	10	126208075	100	4	15.61	52016.22	8.14	44.941	inosine 5 monophosphate dehydrogenase
170	9	126208878	100	1	3.26	54039.78	8.61	7.222	putative malate quinone oxidoreductase
171	10	126207848	100	1	1.40	103746.56	9.53	17.348	serotype specific antigen 1 precursor
172	9	126207929	100	3	7.46	51994.79	8.17	8.309	putative electron transport protein
173	10	126207764	100	3	5.79	73081.29	9.78	15.448	iron regulated outer membrane protein B
174	9	126207929	100	3	7.46	51994.79	8.17	31.222	putative electron transport protein
175	9	126208893	100	3	3.75	93511.73	8.21	28.21	periplasmic nitrate reductase precursor
176	9	126208737	100	1	2.10	47195.81	8.52	10.853	NADH dehydrogenase
177	10	126209294	100	2	6.60	36086.64	9.25	14.709	hypothetical protein APL 1832
178	10	126209294	100	3	11.01	36086.64	9.25	17.545	hypothetical protein APL 1832
179	10	126207750	100	4	23.02	27734.61	9.24	34.298	molybdate binding periplasmic protein precursor
180	10	126209121	100	5	22.31	27931.77	9.15	42.872	putative amino acid ABC transporter binding protein
181	10	126208755	100	3	13.68	23548.30	9.68	73.733	hypothetical protein APL 1289
182	10	126208755	100	3	13.68	23548.30	9.68	50.564	hypothetical protein APL 1289
183	10	126208129	100	2	5.03	39570.96	9.13	19.415	outer membrane protein P2 precursor OMP P2
184	10	126208129	100	2	5.03	39570.96	9.13	18.855	outer membrane protein P2 precursor OMP P2
185	10	126208885	100	2	5.44	39507.21	8.92	8.077	outer membrane protein P5 precursor
186	10	126209314	0	1	3.30	38708.01	9.72	4.179	outer membrane protein P5 precursor OMP P5
187	10	126208885	100	4	11.69	39507.21	8.92	6.051	outer membrane protein P5 precursor
188	10	126209314	0	1	3.30	38708.01	9.72	5.66	outer membrane protein P5 precursor OMP P5
189	10	126209314	100	5	15.66	38708.01	9.72	4.734	outer membrane protein P5 precursor OMP P5
190	10	126208225	100	1	4.71	29881.10	9.49	2.739	penicillin insensitive murein endopeptidase precursor
191	10	126209427	100	3	17.74	22060.36	8.87	8.398	hypothetical protein APL 1973
192	10	141824	25	2	10.69	18447.65	9.50	7.438	hemolysin C
193	10	126209382	100	1	2.03	67941.92	9.35	97.956	biofilm PGA synthesis lipoprotein PgaB precursor
194	10	126207531	100	4	11.01	52973.98	9.46	8.261	phosphatidylserine synthase
195	10	126209183	100	2	11.35	23931.86	10.11	9.844	50S ribosomal protein L1
196	10	126209224	100	2	13.46	22368.94	10.22	6.408	50S ribosomal protein L3
197	10	126209314	100	2	6.59	38708.01	9.72	22.013	outer membrane protein P5 precursor OMP P5
198	10	126209230	100	1	5.11	25880.03	10.56	5.658	30S ribosomal protein S3
199	10	126209247	100	2	12.98	23769.80	10.35	32.602	30S ribosomal protein S4
200	10	126207622	100	6	33.18	24429.50	9.73	52.405	hypothetical protein APL 0134
201	10	126207607	100	4	35.17	20150.67	9.92	54.116	proQ like protein
202	10	126209238	100	3	22.60	18861.12	10.02	20.27	50S ribosomal protein L6
203	10	126209235	100	2	14.53	20341.76	9.88	25.512	50S ribosomal protein L5
204	10	126208082	100	2	14.79	16041.56	9.90	31.366	50S ribosomal protein L13
205	10	126207537	100	4	27.91	18203.46	9.87	31.764	hypothetical protein APL 0049
206	10	126207602	100	3	37.78	9491.17	9.97	39.102	DNA binding protein HU
207	10	126208622	100	2	14.94	18289.27	8.94	7.994	hypothetical protein APL 1152
208	10	126207602	100	1	16.67	9491.17	9.97	6.301	DNA binding protein HU
209	6	126208185	100	5	6.55	111611.50	5.38	21.772	putative methylation subunit type III restriction modification system
210	6	126208185	100	3	3.22	111611.50	5.38	13.925	putative methylation subunit type III restriction modification system
211	6	126208787	100	3	4.89	62702.77	4.81	21.005	phosphoenolpyruvate protein phosphotransferase

212	6	126209366	100	7	10.44	67899.06	4.76	6.596	chaperone protein dnaK
213	6	126208635	100	5	17.18	40646.80	4.86	26.904	hypothetical protein APL 1167
214	6	126208401	100	3	5.19	66662.80	4.94	27.524	chaperone protein HscA like protein
215	6	126208489	100	3	10.47	27662.39	5.63	21.371	deoxyribose phosphate aldolase
216	6	126208420	100	3	22.79	17397.96	4.70	38.349	transcription elongation factor GreA
217	6	126209446	100	1	4.98	25117.05	5.57	34.72	3 oxoacyl acyl carrier protein reductase 3 ketoacyl acyl carrier protein reductase
218	6	126208787	100	4	6.28	62702.77	4.81	7.532	phosphoenolpyruvate protein phosphotransferase
219	6	126208958	100	2	18.52	20732.61	5.71	10.957	fine tangled pili major subunit
220	6	126208059	100	3	5.59	77349.28	5.00	13.79	polyribonucleotide nucleotidyltransferase
221	6	126209101	100	3	16.38	24396.73	4.84	19.436	MTA SAH nucleosidase
222	6	126208764	100	3	16.82	23465.22	5.29	16.308	adenylate kinase
223	6	126209174	100	4	11.36	34748.10	5.16	14.321	ADP L glycero D manno heptose 6 epimerase
224	6	126208541	100	3	22.02	19274.59	4.80	30.465	ferritin like protein 1
225	6	126207522	100	4	12.67	39848.44	4.79	33.433	GTP dependent nucleic acid binding protein EngD
226	6	126208717	100	4	11.25	41144.76	5.01	10.697	phosphoglycerate kinase
227	6	126208567	100	1	3.19	36843.92	5.00	21.903	phosphoribosylformylglycinamidine cyclo ligase
228	6	126208563	100	5	11.79	51649.58	5.29	11.099	aspartate ammonia lyase
229	6	126208348	100	3	3.60	97687.67	5.45	3.448	leucyl tRNA synthetase
230	13	126208432	100	4	5.13	102443.53	5.53	3.876	RTX II toxin determinant A
231	13	126207970	100	1	2.61	44901.09	5.21	6.019	NADP dependent malic enzyme NADP ME
232	6	126209248	100	5	18.24	36517.12	5.26	10.099	DNA directed RNA polymerase alpha chain
233	13	56606845	17	1	3.54	34104.49	5.43	2.201	UDP glucose 4 epimerase Mannheimia haemolytica
234	13	126207930	100	1	4.15	26335.58	7.91	67.806	putative dehydrogenase subunit
235	13	126208127	100	1	3.48	43555.48	5.98	58.717	acetate kinase
236	11	126208250	100	4	7.12	66103.72	5.08	7.818	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2
237	11	126208250	100	4	4.91	66103.72	5.08	11.055	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2
238	11	126208059	100	5	8.95	77349.28	5.00	10.636	polyribonucleotide nucleotidyltransferase
239	11	126208220	100	2	4.69	60113.39	5.08	33.482	30S ribosomal protein S1
240	11	126209110	100	3	10.72	49646.57	5.00	40.184	ATP synthase subunit beta
241	11	126208971	100	2	5.77	47991.97	4.99	36.933	trigger factor
242	11	126208946	100	5	22.13	26683.22	5.25	9.752	hybrid peroxiredoxin HyPrx5
243	11	126208946	100	6	31.15	26683.22	5.25	8.298	hybrid peroxiredoxin HyPrx5
244	11	126209399	100	5	13.16	52290.10	5.20	7.069	glutamine synthetase
245	11	126208253	100	5	15.51	34185.94	5.29	13.927	ribose phosphate pyrophosphokinase
246	11	126208527	100	2	2.46	42443.08	5.17	14.214	3 oxoacyl acyl carrier protein synthase 1
247	11	126208209	100	1	2.45	49090.83	5.25	1.326	antibiotic maturation factor
248	11	126208862	50	3	9.14	43439.30	5.40	7.456	elongation factor Tu
249	11	126209095	100	3	8.20	67158.64	5.54	5.767	glucosamine fructose 6 phosphate aminotransferase isomerizing
250	11	126209095	100	3	7.71	67158.64	5.54	35.571	glucosamine fructose 6 phosphate aminotransferase isomerizing
251	11	126208749	100	1	1.98	62451.12	5.53	21.087	ABC transporter ATP binding protein
252	11	126208276	100	2	4.66	59390.11	5.41	17.284	phosphoenolpyruvate carboxykinase ATP
253	11	126208129	100	5	14.53	39570.96	9.13	15.492	outer membrane protein P2 precursor OMP P2
254	11	126208129	100	6	14.53	39570.96	9.13	16.212	outer membrane protein P2 precursor OMP P2
255	11	126208885	100	2	5.98	39507.21	8.92	26.229	outer membrane protein P5 precursor
256	11	126208885	100	5	14.13	39507.21	8.92	13.946	outer membrane protein P5 precursor
257	11	126209314	0	2	5.77	38708.01	9.72	19.408	outer membrane protein P5 precursor OMP P5
258	11	126208885	100	5	14.13	39507.21	8.92	12.018	outer membrane protein P5 precursor
259	11	126209314	0	2	5.77	38708.01	9.72	17.515	outer membrane protein P5 precursor OMP P5
260	11	126207764	100	4	7.17	73081.29	9.78	9.421	iron regulated outer membrane protein B
261	11	126209314	100	3	10.44	38708.01	9.72	13.865	outer membrane protein P5 precursor OMP P5
262	11	126207675	100	3	7.52	51479.88	6.40	20.823	pyruvate kinase
263	11	126208893	100	4	5.68	93511.73	8.21	41.277	periplasmic nitrate reductase precursor
264	11	126208893	100	2	3.02	93511.73	8.21	6.633	periplasmic nitrate reductase precursor
265	11	126208485	100	5	6.89	95276.65	6.95	13.84	aldehyde alcohol dehydrogenase 2
266	11	126208914	100	3	9.88	36913.55	5.55	37.764	fructose 1 6 bisphosphatase
267	11	126208185	100	2	2.08	111611.50	5.38	47.349	putative methylation subunit type III restriction modification system

268	11	126208510	100	5	6.88	86119.01	5.89	42.944	formate acetyltransferase
269	11	126208637	100	3	27.52	15246.15	6.61	48.774	50S ribosomal protein L9
270	11	126207813	100	1	2.01	43934.26	7.68	32.932	2 octaprenyl 6 methoxyphenol hydroxylase
271	11	126208485	100	2	2.64	95276.65	6.95	16.128	aldehyde alcohol dehydrogenase 2
272	11	126208168	100	2	2.79	91194.05	7.12	11.503	trimethylamine N oxide reductase precursor
273	11	126209138	100	1	1.62	89674.50	7.60	1.137	anaerobic dimethyl sulfoxide reductase chain A precursor
274	11	126209138	100	3	4.22	89674.50	7.60	5.909	anaerobic dimethyl sulfoxide reductase chain A precursor
275	11	126208432	100	6	7.53	102443.53	5.53	6.568	RTX II toxin determinant A
276	11	126207727	100	2	2.54	102431.94	5.41	4.435	preprotein translocase secA subunit
277	11	126208251	100	3	3.39	98856.58	5.57	15.778	pyruvate dehydrogenase E1 component
278	12	126208129	100	5	14.25	39570.96	9.13	8.881	outer membrane protein P2 precursor OMP P2
279	12	126208129	100	1	2.51	39570.96	9.13	11.231	outer membrane protein P2 precursor OMP P2
280	12	126208885	100	2	5.98	39507.21	8.92	12.693	outer membrane protein P5 precursor
281	13	126208432	100	4	4.92	102443.53	5.53	15.218	RTX II toxin determinant A
282	13	126208885	100	1	3.80	39507.21	8.92	12.155	outer membrane protein P5 precursor
283	11	126208250	100	3	4.91	66103.72	5.08	61.643	dihydrolipoyllysine residue acetyltransferase component of pyruvate dehydrogenase complex E2
284	11	126208527	100	1	2.22	42443.08	5.17	65.754	3 oxoacyl acyl carrier protein synthase 1
285	11	126208527	100	1	2.46	42443.08	5.17	1.165	3 oxoacyl acyl carrier protein synthase 1
286	11	126208126	100	4	5.76	76675.17	5.63	8.882	phosphate acetyltransferase
287	11	126208510	100	3	4.42	86119.01	5.89	5.714	formate acetyltransferase
288	11	126208249	100	3	6.75	50520.37	6.11	13.881	dihydrolipoyl dehydrogenase
289	11	126208745	100	2	6.21	46332.25	5.50	13.579	ATP dependent Clp protease ATP binding subunit
290	11	126208249	100	3	7.81	50520.37	6.11	19.039	dihydrolipoyl dehydrogenase
291	11	126208249	100	3	7.81	50520.37	6.11	28.041	dihydrolipoyl dehydrogenase
292	11	126209301	100	2	13.78	27258.83	6.32	21.881	uridine phosphorylase
293	11	126209299	100	1	3.94	37397.37	6.23	2.68	aspartate ammonia ligase
294	11	126208090	100	1	3.05	37600.17	5.76	20.245	phenylalanyl tRNA synthetase alpha chain
295	11	56606845	17	1	3.54	34104.49	5.43	0.533	UDP glucose 4 epimerase Mannheimia haemolytica
296	11	56606845	17	1	3.54	34104.49	5.43	22.206	UDP glucose 4 epimerase Mannheimia haemolytica
297	11	126208181	100	4	11.37	46156.41	5.95	7.988	O acetylhomoserine thiol lyase
298	11	126208163	100	2	6.88	41578.40	6.31	5.795	cystathionine gamma synthase
299	11	126209299	100	4	13.64	37397.37	6.23	3.225	aspartate ammonia ligase

a) the MS# is a continuous numbering of mass spectra

b) this number indicates the preparative gel (Appendix G 5) from which the protein spot was obtained.

c) the probability was calculated using a ProteinLynx Global Server based algorithm which gives very low rates of false positive results.

d) this number indicates the number of peptides that were analyzed by tandem mass spectrometry; each peptide was fragmented and the amino acid sequence was obtained by databank comparison to *A. pleuropneumoniae* serotype 5 strain L20.

e) (number of amino acids in all identified peptides / number of amino acids of the entire protein) x 100

f) the arithmetic mean value of deviations from their calculated mass for all analyzed peptides is the average mass error.

g) the *A. pleuropneumoniae* serotype 5b strain L20 protein database was usually applied for database searches; if no protein was found then the mass spectra were searched against the entire NCBI non redundant protein database; hits obtained different to *A. pleuropneumoniae* serotype 5b strain L20 are mentioned.

G 6.2 MALDI-TOF MS

Results of MALDI-TOF mass spectrometry

MS # [a]	gel # [b]	accession #	mW [Da]	score [c]	expect [d]	matched peptides [e]	protein [f]
M01	14	126208432	102444	149	6.80E-09	24	RTX-II toxin determinant A
M02	14	126208432	102444	193	2.7e-13	27	RTX-II toxin determinant A
M03	14	126208432	102444	149	6.8e-09	13	RTX-II toxin determinant A
M04	14	126208432	102444	255	1.7e-19	46	RTX-II toxin determinant A
M05	14	126208432	102444	234	2.1e-17	25	RTX-II toxin determinant A
M06	14	126208432	102444	205	1.7e-14	34	RTX-II toxin determinant A
M07	14	126208432	102444	210	5.4e-15	29	RTX-II toxin determinant A
M08	14	126208276	59618	103	0.00027	10	phosphoenolpyruvate carboxykinase (ATP)
M09	14	126208862	43553	141	4.3e-08	11	elongation factor Tu
M10	14	126208585	46000	111	4.3e-05	9	enolase
M11	14	126097509	41259	146	1.3e-08	20	phosphoglycerate kinase
M12	14	126208716	39278	251	4.3e-19	18	fructose-bisphosphate aldolase
M13	13	126208432	102444	83	0.026	13	RTX-II toxin determinant A
M14	13	126208432	102444	113	2.7e-05	11	RTX-II toxin determinant A
M15	13	126208251	99028	83	0.028	13	pyruvate dehydrogenase E1 component
M16	13	126208251	99028	84	0.021	12	pyruvate dehydrogenase E1 component
M17	13	126208863	77515	184	2.1e-12	17	elongation factor G
M18	13	126208276	59618	121	4.3e-06	12	phosphoenolpyruvate carboxykinase (ATP)
M19	13	126207608	75970	108	8.5e-05	13	tail-specific protease precursor
M20	13	126209343	109587	86	0.015	8	protease 3 precursor
M21	13	126208168	91536	106	0.00013	14	trimethylamine-N-oxide reductase precursor
M22	13	126207970	45300	90	0.0048	7	NADP-dependent malic enzyme (NADP-ME)
M23	13	126208914	37028	88	0.0078	8	fructose-1,6-bisphosphatase
M24	13	126208914	37028	86	0.014	10	fructose-1,6-bisphosphatase
M25	13	126208158	20918	86	0.014	5	elongation factor P
M26	13	126097696	35824	76	0.12	4	high-affinity zinc uptake system protein ZnuA precursor
M27	13	126207918	35862	84	0.024	7	glyceraldehyde-3-phosphate dehydrogenase
M28	13	126208786	8910	89	0.0069	3	PTS system phosphocarrier protein HPr
M29	13	126208885	39621	115	1.7e-05	8	outer membrane protein P5 precursor
M30	11	126208250	66161	87	0.0098	6	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)
M31	11	126208250	66161	109	6.8e-05	12	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)
M32	11	126208250	66161	82	0.034	13	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)
M33	11	126208250	66161	81	0.044	15	dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex (E2)
M34	11	126208220	60227	106	1.30E-04	11	30S ribosomal protein S1
M35	11	126208276	59618	142	3.4e-08	10	phosphoenolpyruvate carboxykinase (ATP)
M36	11	126208457	73616	116	1.3e-05	13	transketolase 2
M37	11	126208251	99028	94	0.0023	15	pyruvate dehydrogenase E1 component
M38	11	126208251	99028	238	8.5e-18	24	pyruvate dehydrogenase E1 component
M39	11	126208251	99028	87	0.01	21	pyruvate dehydrogenase E1 component
M40	11	126208510	86575	229	6.8e-17	22	formate acetyltransferase
M41	11	126208510	86575	96	0.0012	14	formate acetyltransferase
M42	11	126208510	86575	172	3.4e-11	20	formate acetyltransferase
M43	11	126208918	47625	182	3.4e-12	16	phosphoglucosamine mutase.
M44	11	126208249	50920	124	2.1e-06	11	dihydrolipoyl dehydrogenase
M45	11	126208249	50920	144	2.1e-08	11	dihydrolipoyl dehydrogenase
M46	11	126208755	23548	81	4.10E-02	7	hypothetical protein APL_1289
M47	11	126209224	22369	98	0.00091	10	50S ribosomal protein L3

M48	11	126209109	15216	64	2.10E+00	4	ATP synthase epsilon chain
M49	11	126209294	36144	82	0.036	6	hypothetical protein APL_1832
M50	11	126209183	23932	83	0.028	8	50S ribosomal protein L1
M51	11	126208485	95904	149	6.8e-09	16	aldehyde-alcohol dehydrogenase 2
M52	11	126208485	95904	86	0.015	13	aldehyde-alcohol dehydrogenase 2
M53	11	126208485	95904	129	6.8e-07	17	aldehyde-alcohol dehydrogenase 2
M54	11	126208485	95904	125	1.70E-06	12	aldehyde-alcohol dehydrogenase 2
M55	11	126208485	95904	82	0.032	13	aldehyde-alcohol dehydrogenase 2
M56	11	126208168	91536	99	0.00072	11	trimethylamine-N-oxide reductase precursor
M57	11	126208168	91536	179	6.8e-12	27	trimethylamine-N-oxide reductase precursor
M58	11	126208168	91536	164	2.1e-10	18	trimethylamine-N-oxide reductase precursor
M59	11	126208893	94310	121	4.3e-06	16	periplasmic nitrate reductase precursor
M60	11	126208048	26263	108	8.5e-05	8	30S ribosomal protein S2
M61	11	126207918	35862	84	0.02	5	glyceraldehyde-3-phosphate dehydrogenase
M62	11	126207918	35862	82	0.032	6	glyceraldehyde-3-phosphate dehydrogenase
M63	11	126208594	35777	147	1.1e-08	15	6-phosphofructokinase
M64	11	126208485	95904	117	1.1e-05	21	aldehyde-alcohol dehydrogenase 2
M65	11	126208168	91536	88	0.0081	18	trimethylamine-N-oxide reductase precursor
M66	11	126209237	14146	81	0.041	5	30S ribosomal protein S8
M67	11	126209327	49601	153	2.7e-09	24	Biotin carboxylase
M68	11	33151818	49603	93	0.0028	16	acetyl-CoA carboxylase [Haemophilus ducreyi 35000HP]
M69	11	126209095	67449	230	5.4e-17	26	glucosamine--fructose-6-phosphate aminotransferase (isomerizing)
M70	11	126208862	43553	146	1.3e-08	18	elongation factor Tu
M71	11	126209191	150016	227	1.10E-16	33	DNA-directed RNA polymerase beta chain
M72	11	126209191	150016	102	0.00034	23	DNA-directed RNA polymerase beta chain
M73	11	126209192	157462	92	0.0038	29	DNA-directed RNA polymerase beta' chain
M74	11	126209314	38822	164	2.1e-10	14	Outer membrane protein P5 precursor (OMP P5)
M75	4	126208251	99028	190	5.4e-13	40	pyruvate dehydrogenase E1 component
M76	4	126208348	98201	86	0.012	14	leucyl-tRNA synthetase
M77	4	126208251	99028	69	7.40E-01	12	pyruvate dehydrogenase E1 component
M78	4	126208709	49393	181	4.3e-12	20	glutathione reductase
M79	4	126207911	48805	93	0.0028	9	NADP-specific glutamate dehydrogenase
M80	4	126207532	106437	152	3.4e-09	30	isoleucyl-tRNA synthetase
M81	4	126207532	106437	122	3.4e-06	25	isoleucyl-tRNA synthetase
M82	13	126208276	59618	109	6.9e-05	11	phosphoenolpyruvate carboxykinase ATP
M83	13	126208276	59618	97	0.0011	10	phosphoenolpyruvate carboxykinase ATP
M84	13	126208510	86575	141	4.3e-08	16	formate acetyltransferase
M85	13	126208510	86575	169	6.9e-11	20	formate acetyltransferase
M86	13	126208510	86575	165	1.7e-10	22	formate acetyltransferase
M87	13	126208485	95904	78	0.081	12	aldehyde-alcohol dehydrogenase 2
M88	13	126208485	95904	86	0.014	9	aldehyde-alcohol dehydrogenase 2
M89	13	126208168	91536	192	3.4e-13	26	trimethylamine-N-oxide reductase precursor
M90	13	126208168	91536	90	0.0052	9	trimethylamine-N-oxide reductase precursor
M91	13	126208893	94310	86	0.014	12	periplasmic nitrate reductase precursor
M92	13	126208787	62760	87	0.012	8	phosphoenolpyruvate protein phosphotransferase
M93	13	126208073	59910	113	2.7e-05	16	phosphoglucomutase/phosphomannomutase
M94	13	126208073	59910	121	4.3e-06	18	phosphoglucomutase/phosphomannomutase
M95	13	126208862	43553	147	1.1e-08	24	elongation factor Tu
M96	13	126208862	43553	93	0.0025	12	elongation factor Tu
M97	13	126208752	45904	111	4.3e-05	11	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
M98	13	126208127	43784	88	0.0083	9	acetate kinase
M99	13	126208717	41259	104	0.00022	8	phosphoglycerate kinase
M100	13	126208717	41259	111	4.3e-05	11	phosphoglycerate kinase
M101	13	126207493	40623	95	0.0017	6	aspartate-semialdehyde dehydrogenase
M102	13	126207550	34943	90	0.0061	6	transaldolase
M103	13	126207739	23913	58	9.10E+00	3	manganese superoxide dismutase
M104	13	126208481	47361	92	0.0037	6	HemY-like protein
M105	13	126208247	61028	162	3.4e-10	16	UshA precursor

M106	13	126208607	60548	81	0.04	8	glucose-6-phosphate isomerase
M107	13	126209104	26479	114	2.2e-05	12	putative FKBP-type peptidyl-prolyl cis-trans isomerase
M108	13	126208862	43553	91	0.004	12	elongation factor Tu
M109	13	126208862	43553	122	3.5e-06	13	elongation factor Tu
M110	13	126209294	36144	99	0.00074	11	hypothetical protein APL_1832

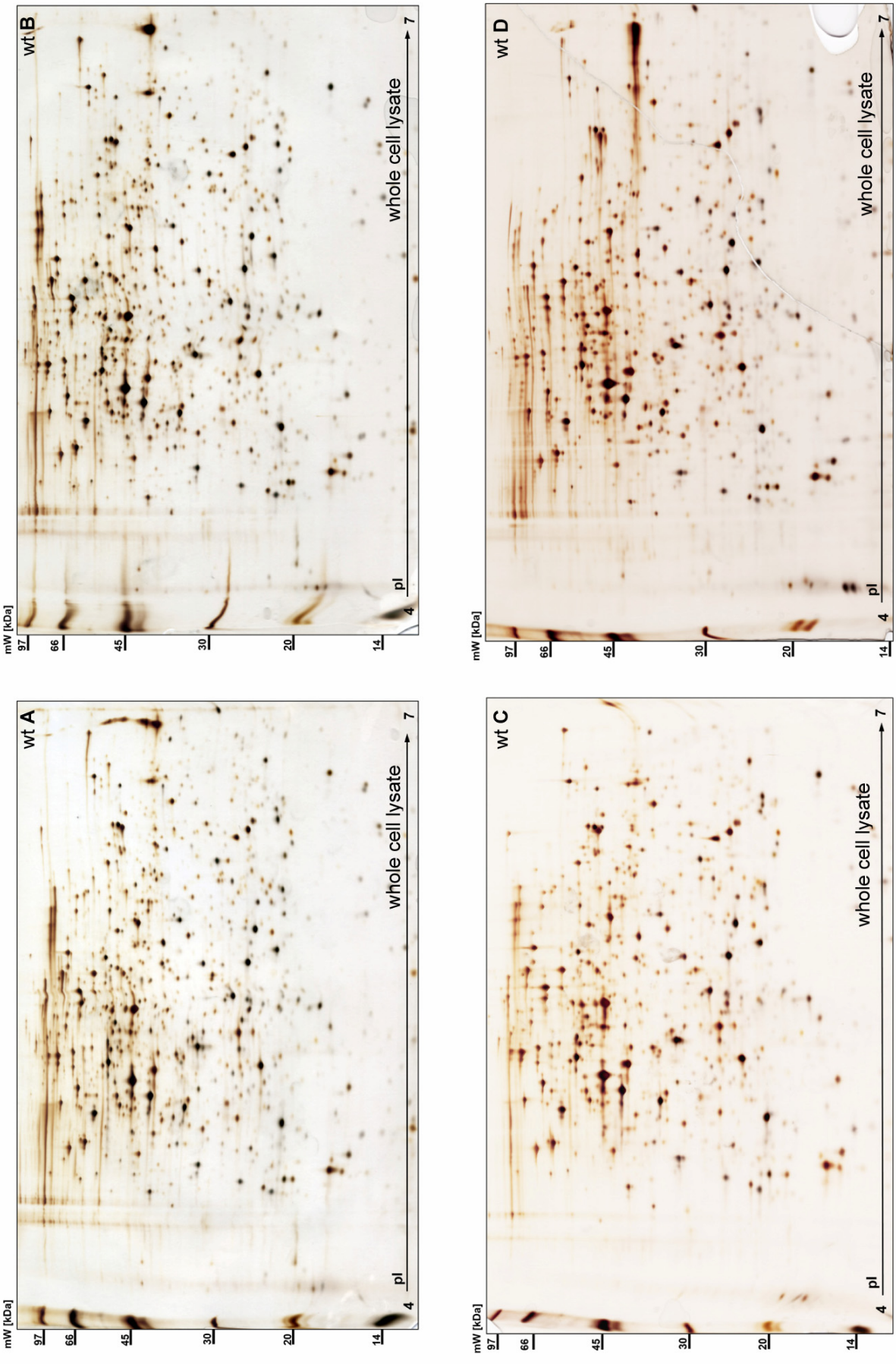
a) the MS# is a continuous numbering of mass spectra and begins in case of MALDI-TOF MS analysis with the capital letter M.
b) this number indicates the preparative gel (Appendix G 5) from which the respective protein spot was obtained.
c) this score was calculated using the matrix science algorithm on the matrix science homepage (http://www.matrixscience.com/search_form_select.html).
d) the expect value was calculated using the matrix science algorithm on the matrix science homepage and represent the probability that the obtained result is false positive. A value below 0.05 indicates statistical significance.
e) the number of peptides of the respective protein that were found by mass spectrometry
f) if not mentioned extra, then the hit was obtained for an *A. pleuropneumoniae* serotype 5 strain L20 protein.

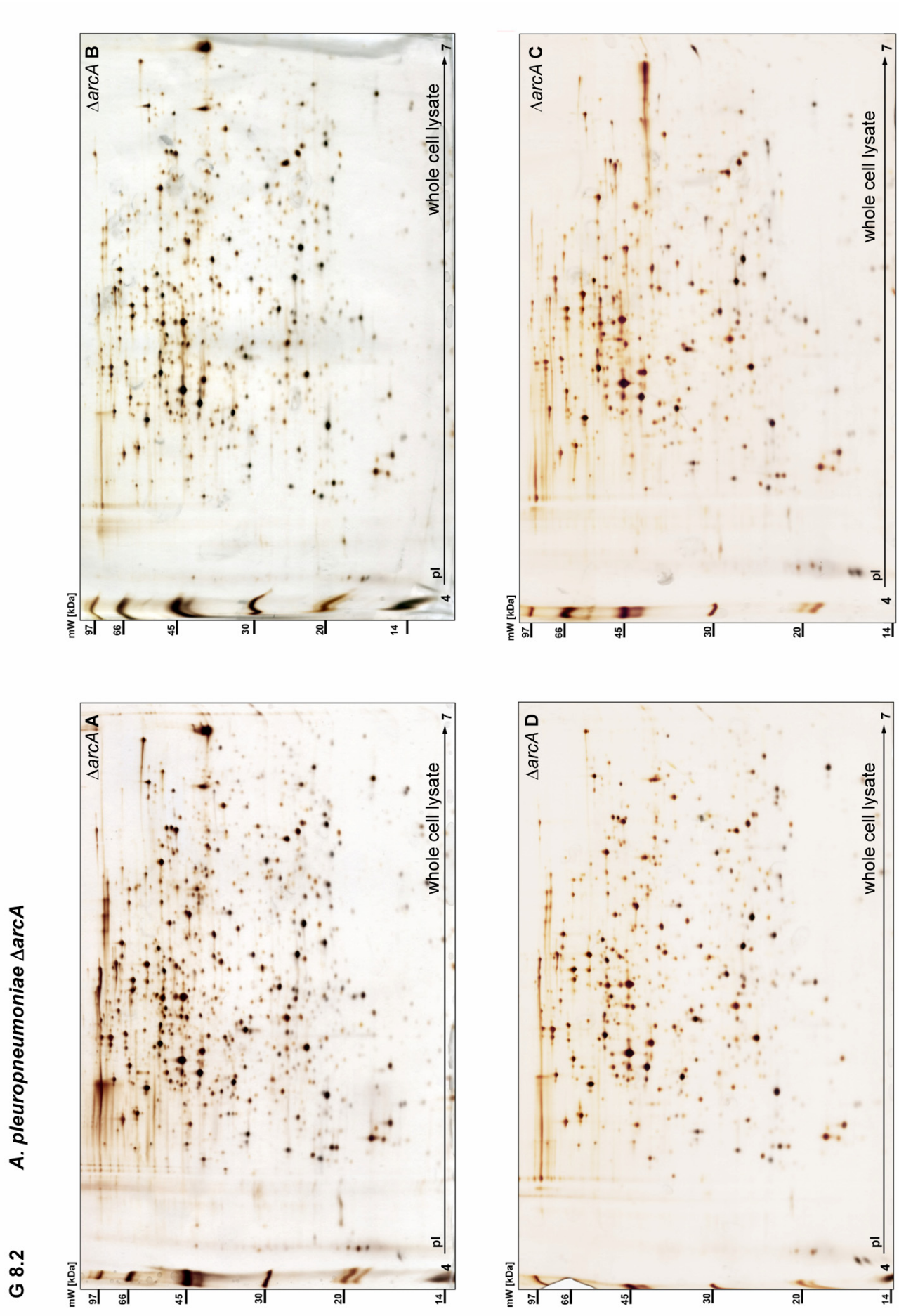
G 7 **Raw data for challenge experiment with *A. pleuropneumoniae* wt and *A. pleuropneumoniae* ΔarcA**

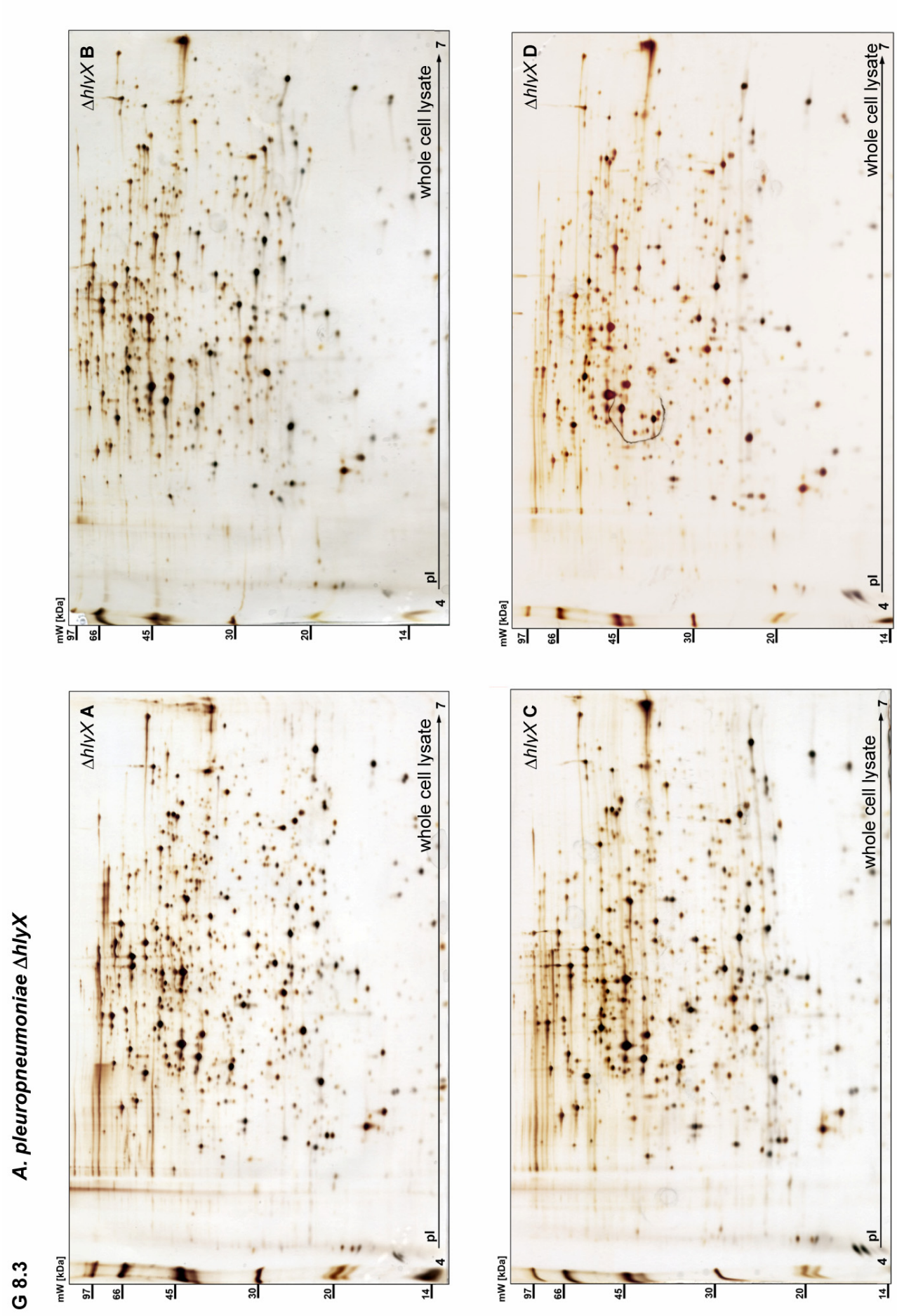
ear tag	group	day of necropsy	clinical score	lung score	ApxIIA / detergent wash ELISA	reisolation from organ samples			
						reisolation score	lung, alterated	lymph node	tonsil
6481 (1)	wt	21	4	16.3	28 / 1:6400	1	++	+	-
6487 (2)	wt	21	1	8.48	33 / 1:200	3	+++	+	-
6406 (3)	wt	21	2	13.53	70 / 1:3200	2	++	+	+
6466 (4)	wt	21	0	0	3 / 1:1600	0	nonexistent	-	-
6492 (5)	wt	21	3	17.6	61 / 1:6400	1	+	+	+
6480 (6)	wt	21	2	7.86	23 / 1:6400	1	+++	+	++
6499 (7)	wt	21	5	5.42	3 / 1:1600	0	+	-	-
6461 (8)	wt	21	7	6.88	10 / 1:400	2	+++	+	+
6403	Δ*arcA*	7	1	0	2 / 0	0	nonexistent	+	++
6483	Δ*arcA*	7	2	2.89	4 / 1:400	1	+++	-	+
6411	Δ*arcA*	7	0	0	1 / 0	0	nonexistent	-	++
6410	Δ*arcA*	7	1	5	1 / 0	0	nonexistent	+	+++
6488	Δ*arcA*	21	0	2.37	88 / 1:6400	0	++	-	+
6473	Δ*arcA*	21	0	0	53 / 1:800	0	nonexistent	-	-
6476	Δ*arcA*	21	2	5.34	22 / 1:1600	0	-	+	-
6463	Δ*arcA*	21	0	2.75	79 / 1:3200	0	+++	+	-

G 8 **Silver staining of *A. pleuropneumoniae* whole cell lysates upon 2D electrophoresis**

G 8.1 ***A. pleuropneumoniae* wt**




G 8.2
A. pleuropneumoniae ΔarcA
ΔarcA A
ΔarcA B
ΔarcA C
ΔarcA D
whole cell lysate
mW [kDa]
97
66
45
30
20
14
pI
4
7


G 8.3
A. pleuropneumoniae ΔhlyX
ΔhlyX A
ΔhlyX B
ΔhlyX C
ΔhlyX D
whole cell lysate
mW [kDa]
97
66
45
30
20
14
pI
4
7

Appendix G 8: Silver staining of 2D gels. *A. pleuropneumoniae* wt, Δ*arcA* and Δ*hlyX* were grown for 4 times independently (A-D) in anaerobic liquid medium. Proteins were precipitated directly from whole cell lysates. The same protein preparations were also used for 2D DIGE later on. In order to proof the preparations and to figure out if the samples were suited for 2D DIGE, each preparation was analyzed by 2D gel electrophoresis. On each gel 50 µg protein was loaded. Proteins were stained with silver nitrate using a modified protocol of the silver staining method of Blum (Rabilloud 1999). By comparing the silver stained gels it is possible to identify some differentially regulated protein spots. However, for this purpose 2D DIGE and spot pattern analysis with DeCyder™ 2D 6.5 software is a much more powerful tool (as shown in “results” of this thesis).

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